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UNITED STATES DEPARTMENT OF AGRICULTURE
BULLETIN No. 810
Contribution from the Bureau of Entomology
L. O. HOWARD, Chief

Washington, D. C. PROFESSIONAL PAPER February 26, 1920

EUROPEAN FOULBROOD

By

G. F. WHITE, Specialist in Insect Diseases

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INTRODUCTION

European foulbrood is an infectious disease of the brood of bees caused by *Bacillus pluton.* It is characterized by the death of brood during its uncapped stage and by the absence of any marked odor. The disorder has a wide distribution and is fairly well known to beekeepers. The losses sustained by the infected apiary vary from a slight weakening of the colonies in some instances to the destruction of all of them in others.

Practical apiarists have determined much concerning the disorder while pursuing their profession. The writer in an earlier paper (15)[1] referred to the nature and extent of the progress that had been made in the study of the disease from the laboratory point of view. The present paper deals with results which have been obtained from a continuation of the work. Among the problems considered are: The resistance of *Bacillus pluton* to heat, drying, sunlight, fermentation, and disinfectants; the effect of the disease on the colony and on the apiary; and the transmission, diagnosis, and prognosis of the disease. Work directly on the treatment of the disease has not been attempted by the writer. Naturally, however, any treatment that is devised, if it is to be efficient and at the same time economical, must be based upon results obtained from the solution of such problems as those which have received attention in these studies.

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[1] Figures in parentheses refer to "Literature cited," p. 34.

Results obtained from a study of the disease in the laboratory and in the experimental apiary form the basis of the discussions contained in the present paper. Since the disease encountered in nature is very similar to the one produced by artificial inoculation, the importance of the studies is at once evident.

The paper[1] will be of interest, it is believed, not only to the apiarist who may wish to apply the facts here determined in the pursuit of his profession, but also to the investigator whose desire primarily is a further study of the disease.

NAME OF THE DISEASE

The term "foulbrood" was quite generally used in the past, as it still frequently is, for the two infectious diseases now known in America as European foulbrood and American foulbrood. In 1885 when Cheshire and Cheyne (4) in England made their studies on foulbrood and described *Bacillus alvei*, evidently they were not convinced that there were two distinct diseases that were being called by the one name foulbrood. The disease studied by them is the one which is the subject of discussion in the present paper. In the names for the two diseases it will be observed that the word "foulbrood" is retained in both instances. To this "European" is added for the disease on which early laboratory studies were made by these Europeans (Cheshire and Cheyne).

Dr. William R. Howard (6), of Texas, in 1900, worked for a brief period with this disease, reached the conclusion that it was a new one, and referred to it by the names "New York bee disease," or "black brood." Work by Moore and White (11) in 1902 showed that the disease was not new, but was the foulbrood studied by Cheshire and Cheyne (4). The names "New York bee disease," or "black brood," therefore, were superfluous, and as their use would have added to the confusion that already existed they were discarded. Beekeepers, entomologists, and pathologists, as a rule, are more or less familiar with the terms "foulbrood" and "*Bacillus alvei*." Usually, however, the ropy foulbrood—American foulbrood—is the one that is thought of, and the one that frequently has been associated in the literature with *Bacillus alvei.* This is unfortunate. While *B. alvei* is not the cause of any bee disease, it occurs very frequently with European foulbrood and is found only seldom in the ropy disease. In using the names European foulbrood and American foulbrood it is possible, however, to avoid confusion by bearing well in mind the history of the disease.

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[1] The present studies are similar to those made by the writer on sacbrood (17), Nosema-disease (18), and American foulbrood (19). A reference to these papers may be found helpful where the discussions in the present one are especially brief. The investigations were completed in September, 1916, and the paper was submitted for publication in October, 1918.

HEALTHY LARVÆ OF THE AGE AT WHICH THEY DIE OF EUROPEAN FOULBROOD

Bees dying of European foulbrood do so during the larval stage.[1] Death may take place at any time from the fourth day of larval life to pupation. For convenience of description the brood of the age at which death from European foulbrood occurs is placed here in three groups. Groups 1 and 2 include the uncapped and group 3 the capped larvæ.

GROUP 1

The youngest larva (Pl. II, D, G) that dies of European foulbrood practically covers the bottom of the cell. It lies either on its right or its left side, with its dorsal portion extending to the lateral walls of the cell. Its form is **C** shaped with the anterior and posterior extremities almost together. Its color is bluish white with a glistening surface, presenting a pearly appearance. The body is more or less opaque, due largely to the adipose tissue. Folds and furrows divide the surface into segments. In health these are quite prominent and the entire larva is turgid in appearance.

With the unaided eye spiracles and tracheæ can be seen with difficulty, but by slight magnification they are readily observed. Most of the tracheæ, appearing as white lines, extend either dorsally or ventrally on the lateral side of the larva, but a distinct chain connecting them will be observed to extend at right angles to these.

GROUP 2

Healthy larvæ (Pl. III, D, G) slightly older than those described in Group 1 constitute Group 2. The larva now completely fills the bottom of the cell. The dorsal side pressing against the lateral side walls of the cell causes the contour of the body to be in general hexagonal. The tracheæ are seen less easily than in younger larvæ, while the color, glistening appearance, prominence of segments, and turgidity are similar to those of the younger larvæ described in Group 1.

By turning the larva so that its dorsal surface may be brought into view (Pl. III, A) there is observed a more or less transparent narrow area along the dorsal median line extending nearly the length of the body. The contents of the stomach may be seen through this area. The color of the mass is due chiefly to the presence of pollen. It is usually some shade of yellow. The median area presents in its appearance a sharp contrast to the bluish-white, opaque portions on either side of it. Similar appearances are to be noted in the larvæ of Group 1.

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[1] The term larvæ as used in the present paper applies to the prepupæ as well as to earlier stages of the brood.

The larva removed from the cell performs only slight movements, lies partly coiled, and is more or less turgid. The segments are prominent. When the body wall is torn there flows from the ruptured wall the clear larval blood, in which are suspended often fat and other tissue cells which give to it a somewhat milky appearance. The stomach, a transparent tube easily torn into segments, contains a mass of partially digested food, pollen constituting usually a conspicuous portion of it.

GROUP 3

Group 3 consists of capped larvæ. These are, therefore, larger than those described in Groups 1 and 2. In the group are included the larvæ which have spun a cocoon as well as those which have not. An endwise position in the cell may or may not have been assumed. The larvæ are seen in various positions. Not infrequently some portion of the dorsal surface is turned toward the observer, the narrow, median, transparent area being in evidence as in younger larvæ. Healthy larvæ occupying an endwise position are described in papers on sacbrood and American foulbrood (17, 19) and will not be referred to further at this time.

SYMPTOMS

In European foulbrood, as in other brood diseases, the colony as a whole and not the individual bee should be considered as the unit in the discussion of the symptoms of the disease. The description of the symptoms recorded in the present paper is based chiefly upon observations made on the disease produced through artificial inoculations. In making the studies in the experimental apiary observations made by beekeepers have been duplicated and new facts determined. It has been possible also to locate errors which have been made in discussions of symptoms of the disease.

GENERAL SYMPTOMS FROM A CASUAL EXAMINATION

Death of brood during the feeding stage, in uncapped cells, is a characteristic of European foulbrood. The brood nest in the disease usually presents an irregular appearance, capped cells and uncapped ones being found scattered irregularly over the brood frames, giving to them the "pepper box" appearance (Pl. I) often referred to by beekeepers, a condition noticeable when the disease is fairly well advanced in the colony.

The dead larvæ lose their pearly whiteness and assume a yellowish color, later becoming brownish. This deepens often to a dark brown. The decaying remains are not characteristically ropy, as in American foulbrood. Marked viscidity is usually absent. When it is present the decaying mass can be drawn into threads but to a less extent than in the ropy disease. In advanced cases the disease may be accompanied by an odor, but in the writer's experience this never has been marked and never offensive.

As the disease in the colony advances, weakness becomes a symptom. In severe cases queenlessness may result from the infection. This, however, is by no means the rule.

SYMPTOMS MANIFESTED BY INDIVIDUAL LARVÆ SICK OR DEAD OF EUROPEAN FOULBROOD

Evidences of European foulbrood in the individual larvæ appear before and after death. The colony symptoms used most frequently in the diagnosis of the disease are largely post-mortem appearances of larvæ. Of much interest and frequently of considerable diagnostic value are the symptoms manifest by larvæ sick but not dead of the disease. For convenience in the description of the appearances of the sick or dead larvæ, the grouping used in describing the healthy larvæ (p. 3) is followed. The appearances of affected larvæ both living and dead are, of course, changing constantly. A description which is correct for one day or hour, it should be realized, is not likely to be entirely correct for the next.

GROUP 1

The youngest larvæ manifesting symptoms of European foulbrood are approximately 4 days old (Pl. II, A, B, C, E, F, H, I). In many cases at this stage of the disease a peristalsis-like movement of the body is marked and is readily observed by the unaided eye, but in others no such bodily movements are observed. The diseased larvæ at the time may be more transparent (Pl. II, B, H) than healthy ones of the same size. In such larvæ the tracheæ are quite prominent and more readily seen than in healthy ones. Occasionally numerous minute opaque areas are observed in these more transparent larvæ, giving to them a punctate appearance. Very often, however, this sign is not present. In many instances, indeed, no distinct symptom is observed until the larva approaches death. (Pl. II, A).

Larvæ (Pl. II, A, B, C, E, H, I) of this group dying or just dead of the disease lose their marked glistening appearance; their pearly whiteness gives way to a yellowish tint; the turgidity seen in healthy larvæ is diminished in the sick; and the folds and furrows indicating the segments of the body become less prominent. As the process of decay advances the yellowish hue changes, the color assuming a brownish tone. The segmental markings are less prominent, while the tracheæ often become quite distinct, appearing as white lines contrasted with the darker color of the larval remains (Pl. II, B). Not infrequently at this time there will be seen a chitinous envelope containing a watery-looking fluid in which is the larva proper (Pl. II, C; Pl. IV, A). The decay proceeds and the drying becomes evident. The larval mass settling upon the concave bottom of the cell causes the upper surface of the mass to be depressed about the center. At this stage the tracheæ not infrequently are seen distinctly in the drying mass. When the larval remains become dry they are known as the scale (Pl. II, F). The scales do not adhere closely to the cell and when removed are found to be thin and more or less circular in outline. They are convex and smooth on the side which was in contact with the bottom of the cell while the opposite surface—the one which, while in the cell, was toward the observer—is slightly roughened and concave.

GROUP 2

Larvæ (Pl. III) showing symptoms of European foulbrood and classed in this group have reached a sufficient size to fill the deepest third or more of the cell. The yellowish tint appears in contrast to the bluish white of the healthy larvæ (Pl. III, D, G). Increased movement may or may not be observed. Before and after death the remains may assume one of a number of positions in the cell. Not infrequently a portion of the dorsal surface is turned toward the observer (Pl. III, B). Usually through the transparent area along the median dorsal line a whitish or yellowish-white mass is to be observed. This mass is within the stomach of the larva and contains a large amount of bacterial growth (Pl. VIII, a, b, c) consisting very largely of *Bacillus pluton*. Often before death this mass is seen to move within the stomach in response to the peristalsis-like movements of the body of the larva.

At the time of death the larva usually occupies some unnatural position, being more or less curled up and lying upon the floor of the cell (Pl. III, C, E, F, H, I). Lessened turgidity, a relative dullness of the surface appearance, and a yellowish tint are present. Not infrequently the two ends of the larva are directed more or less toward the bottom of the cell and some portion of the dorsal surface is toward the opening of it (Pl. III, E, H, I). Among the dead larvæ will be found some with one end directed toward the bottom, and the other toward the mouth of the cell, the body occupying a more or less spiral position against the side walls and floor of the cell (Pl. III, F).

Later the dead larval remains assume a brownish tint which deepens to varying shades as decay continues and drying takes place. During the early part of the decay, the firmness of the body wall permits the removal of the larva intact from the cell. Later, however, it offers but little resistance and is easily ruptured. The decaying mass before drying often attains a certain amount of viscidity. Sometimes it is of a doughy consistency, at other times it is purulent or sputumlike, while at times it assumes a viscidity that will permit of its being drawn out to the extent of an inch or more. When the larval mass becomes dry it forms an irregular scale, usually brown in color, lying on the floor or side wall of the cell or both, but not adhering closely to them.

GROUP 3

A larva dying of European foulbrood after being capped may be found occupying one of many positions within the cell (Pl. IV, C, D, E; Pl. V, D, E, F, G, H). Dying before the two-day quiescent period that precedes pupation, the remains during decay and as a scale resemble in many respects those of larvæ described in group 2. The dry scales occupy usually an irregular position on the floor of the cell (Pl. IV, F, G). Dying during the two-day quiescent period, however, the scales (Pl. V, F, I) resemble very much those of larvæ dying at the same age of American foulbrood. The larval mass assumes the brownish hue which deepens as the decay advances, reaching a dark brown. Viscidity is present in the decaying larval mass, but the extent to which the decaying material may be drawn out is less than in American foulbrood. The scale is less brittle and more rubberlike.

At no time has the writer observed pupæ dead of European foulbrood. If they die of the disease it is a rare occurrence.

The removal of larvæ sick or dead of the disease is accomplished to a greater or less degree by adult workers. The larvæ are either partially or entirely removed. This is usually done piecemeal. In an infected colony will be found, therefore, the remains of larvæ of different ages (Pl. IV, B) and (Pl. V, A) in varying numbers.

ETIOLOGY

PREDISPOSING CAUSES

Age.—Infection in European foulbrood takes place during the feeding stage and at some time after the first day of larval life, the larvæ being more often 2 days of age, or older. Death takes place somewhat more than 2 days from the time of infection. As a rule, therefore, a larva has passed its fourth day of larval life before death from European foulbrood occurs. From this age to pupation larvæ may die of the disease. The writer has not encountered death among brood which has reached the pupal stage. Adult bees are not susceptible to infection.

Sex.—Worker, drone, and queen larvæ are all susceptible to infection with *Bacillus pluton* and any of these may die of European foulbrood.

Race.—Complete immunity from European foulbrood has not been found among the races of bees studied. Experimental work recorded in the present paper involved the use of at least five colonies of "tested Italians," two of "tested Carniolans," and two of "tested Caucasians." For the most part the bees used were "untested Italians," but among the colonies were a few common blacks. In all these strains the disease was readily produced through experimental inoculation. The examination of numerous samples of diseased brood received from beekeepers throughout the United States suggests that all races commonly kept by American beekepeers are susceptible to European foulbrood. The relative immunity of the different races has not been demonstrated by the studies. These facts, however, do not dispute the observation by practical beekeepers that some strains of bees show a greater colony resistance than others.

Climate.—From reports of studies made in Austria by Muck (12), in Denmark by Bahr (1), in England by Cheshire and Cheyne (4), in Germany by Zander (20), and in Switzerland by Burri (3), it is clearly evident that the disease discussed in the present paper occurs in these different countries. It has been encountered also in many sections of the United States and Canada. This distribution shows that the infection can exist under a variety of climatic conditions. The practical import of the fact is that the presence of European foulbrood in any locality can not be attributed entirely to the climate of the region.

Season.—Beekeepers have observed that European foulbrood occurs with greatest severity before midsummer rather than later in the season. The disease, it has been shown experimentally, can be produced, however, at any season of the year at which brood is being reared. Its severity at any given season is to be attributed, therefore, to environmental conditions rather than to the difference in the susceptibility of larvæ during the different seasons.

Food.—As in American foulbrood it is found that the cause of the disease in the colony is governed very little if at all by the quality of food gathered by bees. Indirectly, however, the quantity present in the hive or obtainable often does influence its course materially.

EXCITING CAUSE

That *Bacillus alvei* may be present in large numbers in brood dead of foulbrood was demonstrated by Cheshire and Cheyne (4) in 1885.

For a decade and a half following the observation the belief was quite general that this bacterium was the exciting cause of a bee disease. The view was then seriously challenged. In 1906 the only positive conclusion in regard to the relation between European foulbrood and *Bacillus alvei* that could be drawn by the writer (13) was that this species occurs in brood dead of the disease.

William R. Howard (6), of Texas, after a brief study of the disease reported in 1900 the presence of an organism which he called *Bacillus millii.* He cultivated the species apparently with ease. In 1904 Bahr (1) in Denmark found a small oval bacterium in a brood disease in which larvæ dying in uncapped cells are yellowish in color and not ropy in consistency. Burri (3) in 1906 encountered in his studies on the brood diseases a small bacterium which he referred to as *guntheri*-forms. The species was cultured and compared with *Bacterium guntheri* and found to be somewhat different. In 1907 Maassen (7) obtained from brood material cultures of a species which he named *Streptococcus apis.* White (14) in 1908 reported the presence of a small organism in European foulbrood which had refused to grow on artificial media. The species was not the one, therefore, with which the investigators just referred to had worked. That this organism might be the exciting cause of the disease was noted. Pending more information regarding it, the species was not given a name but was referred to as bacillus " Y." That this species bears a direct etiological relation to the disease was demonstrated in 1912 by the writer (15) and the name *Bacillus pluton* was then given to it.

As the cultivation of *Bacillus pluton* on artificial media had not been accomplished the conclusion that it is the exciting cause of European foulbrood was arrived at by eliminating all other possible agencies. The observations furnishing the proof appear in an earlier paper (15). By demonstrating *Bacillus pluton* to be the cause of the disease, *Bacillus alvei*, *Streptococcus apis*, *Bacterium eurydice*, and *Bacillus orpheus*, and still other species occasionally encountered, were thereby proven to be secondary invaders.

To eliminate the possibility of a filterable virus in European foulbrood 10 colonies were inoculated with filtrates obtained from aqueous suspensions of brood sick and dead of the disease. In six instances the Berkefeld N filter was used and in four the Pasteur-Chamberland F was employed. In no case was the disease produced. Studies recorded in the present paper on the resistance of *Bacillus pluton* to heating, drying, fermentation, and disinfectants show that when the virus of the disease is not destroyed this species is still alive. This fact is further evidence in support of the conclusion that the species *Bacillus pluton* is the virus of the disease.

BACILLUS PLUTON

An artificial medium for the cultivation of *Bacillus pluton* has not yet been devised. To accomplish this may or may not be a particularly difficult task. The media ordinarily used in the laboratory are not suitable. Bee-larvæ agar, brood-filtrate media, egg-yolk-suspension agar (19), and combinations of these have not thus far proved sufficient for the purpose. The species is an unusual one. The generic classification has not been determined definitely and this may not be possible until the proper condition for the artificial cultivation of the species has been supplied.

The morphology of *Bacillus pluton* is somewhat variable. In very early infection its form is that of a short rod in pairs or in chains, or possibly of a coccus with the individuals similarly arranged (fig. 1; Pl. VII, B). The length is then equal to or somewhat greater than the breadth. In slightly later stages of infection the predominating form is that of a lancet-shaped coccus (fig. 1; Pl. VII, A), and in late stages this form is present almost exclusively. The lancet form occurs singly, varying greatly in size and having a length which approximates twice the width. The length is more often less than 1 μ than greater. The organism colors uniformly with the aniline stains, stains with iron hematoxylin, and is gram-positive. It does not form spores. This is evidenced by the microscopic appearance and also by the thermal death point of the species. Its resistance to drying, disinfectants, and other environments is discussed later in the present paper.

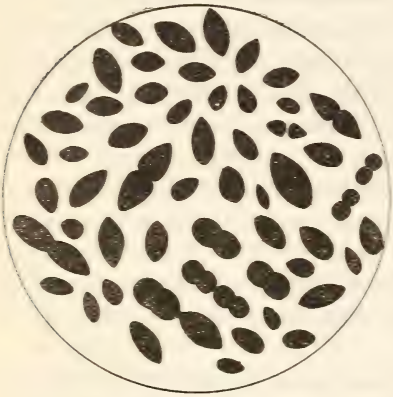

FIG. 1.—*Bacillus pluton.*

Seven rabbits inoculated, six subcutaneously and one intraperitoneally, with a suspension of larvæ dead of European foulbrood proved to be refractory. Only a slight rise of temperature followed the inoculations and the weight was not materially affected. Six guinea pigs inoculated subcutaneously with similar material proved not to be susceptible to infection with the species. Four pigeons inoculated in the pectoral muscles and two white rats inoculated subcutaneously also proved refractory. In none of these inoculated animals were there any lesions of particular note produced.

Growth of *Bacillus pluton* in the infected larva begins close to the surface of the peritrophic membrane (Pl. VII, I) in contact with the food of the larva. As growth continues the bacterial mass extends toward the center of the lumen of the peritrophic sac (Pl. VII, K), finally filling it more or less completely (Pl. VII, J). The growth does not always take place uniformly along the peritrophic membrane (Pl. VII, J), nor does it extend beyond it (Pl. VII, I, J, K), but is inclosed within the sac, the tissues of the larvæ not being reached. The multiplication of the organism after the death of the host, if, indeed, it takes place at all, is limited.

Secondary invaders, chiefly *Bacillus alvei*, *Bacterium eurydice*, *Streptococcus apis*, and occasionally *Bacillus orpheus*, and a few others, are encountered at various stages of the disease and during the decay of the larva. During the life of the larva these species also remain within the peritrophic sac.

BACILLUS ALVEI

Bacillus alvei (fig. 2; Pl. VII, D, F) is present very frequently and in very large numbers in larvæ dead of European foulbrood. The species was well described by Cheyne (4). Descriptions may be found elsewhere also (11, 13). It is readily recognized and may be differentiated easily from other spore-producing species occasionally encountered in the diseased brood.

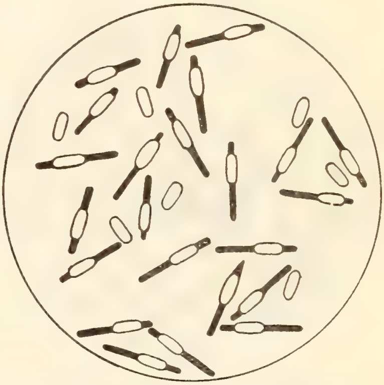

Fig. 2.—*Bacillus alvei*. Spores free from and others within rods.

Bacillus alvei is not the active cause of any bee disease. It seems probable, however, that it plays a rôle in European foulbrood, but the extent is not fully known. The species is present usually, if not invariably, in large numbers in the rubberlike scales (Pl. V, F, I), which resemble so much those of American foulbrood. The decayed larval mass, which forms the scale, before becoming dry is ropy in consistency similar to that of American foulbrood but to a less degree. It seems probable that this ropiness is due more or less directly to *Bacillus alvei*. On account of this viscidity the decaying mass, as well as the scales, are removed with greater difficulty than are most larvæ dead of European foulbrood. The result, as often observed, is that these brown viscid decaying larvæ or the rubberlike scales resulting from them are the only evidence that European foulbrood is present in the colony.

While *Bacillus pluton* in such larval masses and scales is often difficult to detect microscopically, its presence can be demonstrated through the experimental inoculation of healthy larvæ. Inasmuch as *Bacillus pluton* will live for a considerable period in the scales, it seems quite probable that the disease might in some cases be carried over for months or even over winter through the medium of these rubberlike scales.

It is of interest to know that the amount of disease resulting immediately from inoculations in which scale material is used is much less than when larvæ recently dead of the disease are used. This is true also of dead larvæ stored in Petri dishes compared with smears allowed to dry immediately from larvæ recently dead of the disease. These facts indicate a possible deleterious effect on *Bacillus pluton* of the secondary invaders multiplying in the decaying larvæ.

STREPTOCOCCUS APIS

It is most probable that *Streptococcus apis* is the species that was isolated from diseased brood by Burri (3) and referred to by him in 1906 as "*güntheri*-forms." Maassen described it in 1908 (8). The organism grows well at incubator, room, and refrigerator temperatures in most of the media ordinarily used in the laboratory. Its cultural characteristics suggest the micrococci rather than the streptococci. Confusion in some of the earlier investigations was due evidently to the resemblance of *Streptococcus apis* and *Bacillus pluton* morphologically. To this fact is due the chief interest in the species *Streptococcus apis*. When encountered in larvæ dead of European foulbrood it can be identified readily by culturing. The generic position of this species should be considered as being not altogether certain.

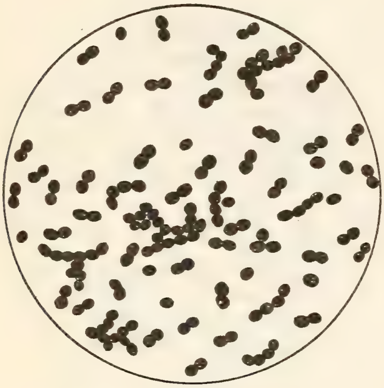

FIG. 3.—*Streptococcus apis.*

Occurrence.—*Streptococcus apis* is occasionally encountered in larvæ dead of European foulbrood and often is present in large numbers.

Morphology.—It is more or less spherical (fig. 3; Pl. VII, E), occurring singly and in pairs with occasionally a chain of 2 or more pairs when grown in liquid media. In larval remains not infrequently the ends may be somewhat pointed.

Staining properties.—It colors uniformly and readily with the common stains, and retains the stain after Gram's method.

Glucose agar plate.—Within a day growth is visible. Colonies never become large. Surface colonies are usually less than 2 mm. They are circular with uniform outline and a well-defined border, are grayish by reflected and bluish by transmitted light, are smooth and convex, are moist and glistening in appearance, and are friable in consistency. When magnified the surface colonies appear light brown in color, and granular in structure, the density decreasing from the center to the periphery. Deep colonies appear dense, dark brown, and coarsely granular. They are in general lenticular to oval but are sometimes almost spherical in form.

Glucose gelatin plate.—At refrigerator temperature and within 3 days, the surface colonies begin to liquefy the gelatin, each liquefied area appearing somewhat as a minute drop of water.

Agar slant.—In one day numerous gray colonies cover the inoculated surface.

Bouillon.—Within a day the medium is uniformly and moderately clouded.

Fermentation.—In glucose, lactose, saccharose, levulose, maltose, and mannite bouillons, a uniform clouding of the media occurs. The growth takes place in both arms of the tube, but is heavier in the open one. Considerable acidity, but no gas, is produced.

Milk.—Milk is rapidly coagulated. Digestion of the coagulum follows. In from 3 to 5 days more than one-half has been changed. Within 24 hours the color is discharged in litmus milk, except at the top of the medium. In other respects it is like the plain milk.

Potato.—No visible growth. That growth in the potato water takes place is confirmed by microscopic examination.

Gelatin stab.—Liquefaction along the line of puncture is appreciable after one day. In four days a cylinder of liquefied gelatin 1 cm. in diameter surrounds the original line of puncture and soon extends to the walls of the tube.

Pathogenesis.—No disease results when the brood of bees is fed cultures of *Streptococcus apis* either by the direct or indirect method. A rabbit and two guinea pigs inoculated with a pure culture of *Streptococcus apis* were not susceptible to infection with the species.

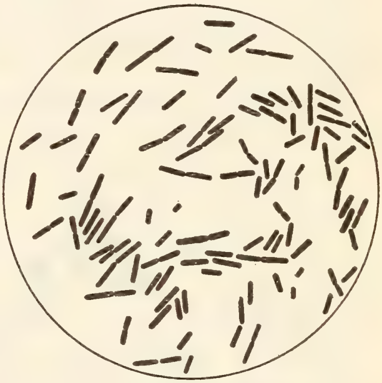

FIG. 4.—*Bacterium eurydice.*

BACTERIUM EURYDICE

The presence of this species in European foulbrood was pointed out by the writer in an earlier publication (15). Among the secondary invaders in larvæ infected with *Bacillus pluton*, *Bacterium eurydice* is one of the earliest to be found. It is often present in considerable numbers. In plating for the species the stomach contents from larvæ sick, but not dead, of the disease should be used. In studying this species cultures were isolated which in some respects differed from it. Whether these are different species or belong to a group of which *Bacterium eurydice* is a representative has not been definitely determined.

To isolate *Bacterium eurydice* the plating has been done with glucose agar. Incubation must be carried out at room temperature. Growth of the species is always slow and never luxuriant. Under favorable conditions colonies are visible after one day. To preserve cultures they must be renewed frequently.

Occurrence.—*Bacterium eurydice* is frequently present in larvæ sick or recently dead of European foulbrood.

Glucose agar plate.—To the naked eye the surface colonies are slightly convex, smooth, and glistening. They are from 1 to 2 mm. in diameter, circular and uniform in outline. The color is bluish by transmitted and grayish by reflected light. Under a two-thirds objective they are a light brown, and are finely granular near the periphery, but more coarsely granular near the center.

Morphology.—The rod (fig. 4; Pl. VII, C) is small and slender with slightly rounded ends, occurring usually in pairs or singly. It is nonmotile and no spores are produced.

Staining properties.—It is stained easily and uniformly with the ordinary aniline stains and is Gram-negative.

Oxygen requirements—Growth is better in the presence of air than in anaerobic conditions.

Bouillon.—Growth takes place slowly, producing a uniform cloudiness with no pellicle. After a week or more a somewhat viscid sediment is present.

Sugars.—Growth in the sugar media is slow, variable, and never luxuriant. Both arms may be clouded. Glucose or levulose when added improves a medium. Fermentation with gas does not take place in any of the sugars. A noticeable amount of acid is formed when glucose and levulose are used, the other sugars being less affected. A 1 per cent honey solution supports a moderate growth. Brood filtrate as a rule improves media.

Milk.—In plain and litmus milk no changes are visible.

Potato.—Growth on potato is slow. When present, the culture is for the most part grayish in color.

Gelatin stab.—A bluish gray growth appears slowly along the line of inoculation. No liquefaction follows.

Pathogenesis.—No ill results are observed when cultures of *Bacterium eurydice* are fed to healthy colonies of bees. A rabbit inoculated subcutaneously with a pure culture proved to be refractory.

BACILLUS ORPHEUS

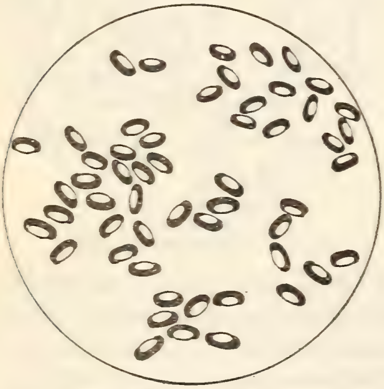

FIG. 5.—*Bacillus orpheus:* Spore formation.

The name *Bacillus orpheus* was given to an interesting species occasionally encountered in European foulbrood (15). In one instance the species was found very widely distributed in an apiary in which heavy losses were being sustained from the disease. In this case the dead larvæ when dry were stonelike in character, the petrified remains breaking like so much marble. Usually the species is met with in a less number of the affected larvæ. It can be readily identified from its morphology and cultural characteristics. A description of the species has been made by McCray (9). An organism similar to *B. orpheus* in many respects has been described by Laubach (5) and named *Bacillus laterosporus.*

The organism is a motile spore-bearing rod with a few peritrichic flagella. Spore formation begins in a few hours on the surface of the agar at incubator temperature, the rod swelling toward the center and becoming fusiform. Soon, as determined from stained preparations, the spore is seen occupying one side of the rod with the protoplasm distributed along the opposite side and the two ends (fig. 5; Pl. VII, H). The rod, together with the spore within it, measures about 2.4μ in length and 1.2μ in width. This relation of spore and rod persists in cultures on a solid medium for a long period, especially at room temperature. Good growth, no gas, and only slight changes in reaction occur in the sugar media. A slight coagulum forms in the milk which is slowly digested. Gelatin is rapidly fluidified.

Bacillus orpheus is not pathogenic for the brood of bees when inoculated by feeding either by the direct or indirect method. Silkworm larvæ succumb following inoculation by feeding and also by puncture.

TECHNIQUE[1]

Artificial conditions for the successful cultivation of *Bacillus pluton* have not yet been obtained. That this can be achieved by further study is not at all improbable. Without having accomplished this, it has been possible, however, to make the studies on the biology of the parasite that were most desired. This was done through experimental work, using the larvæ of bees. The inoculations were made by feeding a suspension of the organism in sugar sirup.

Two methods were employed in making the feedings, which will be referred to here as (*a*) the indirect method, in which the colony is inoculated, and (*b*) the direct method, in which only a few larvæ are inoculated. Cane sugar and water were used in preparing the sirup in the proportion approximately of 3 to 2. This solution was then brought to the boiling point.

From 5 to 10 diseased larvæ furnish sufficient infective material when the indirect method is followed. These, after being picked from the brood frame, are thoroughly crushed, added to about 300 c. c. of the cooled sirup, and fed to a colony.[2] When the suspension contains the living virus, symptoms of European foulbrood appear in 3 days following the inoculation. The earliest evidence of disease is manifested by sick rather than dead larvæ (p. 5). Often fragments of larvæ (Pl. IV, B) are found upon examination of the brood nest.

In the direct method *Bacillus pluton* is taken from the stomachs of infected bees. Sick rather than dead larvæ are preferred for obtaining the virus free from the body tissues. By the use of dissecting needles and with a little care the stomach contents (Pl. VIII) can be pulled out of the blind end of the organ (15). The virus-containing material thus obtained is triturated with water and the aqueous suspension is added to sirup. The suspension of *Bacillus pluton* in a thin sirup is used in making the inoculation. Larvæ about 2 days old are especially desirable for the direct method. The inoculation is made by adding a small amount of the suspension to the food of the larvæ by means of a capillary pipette made from glass tubing of small bore. Care must be exercised in thus feeding the larvæ. Too much of the suspension will often float the larva. There is danger also that it will be changed in position mechanically by means of the feeding pipette. In either event the chances are that such larvæ will be removed subsequently by the bees. Considerable larval food already in the cell is advantageous. This method has proved to be especially useful in much of the experimental work recorded in the present paper. It has the advantage of being both economical as to the number of colonies needed, and definite. During the third day following the hour of inoculation symptoms of European foulbrood will be observed if infection is produced. By the fourth day frequently all of the infected larvæ will have been removed by the bees. Symptoms of European foulbrood infection manifested by larvæ sick rather than dead have proved to be especially useful for experimental purposes in these studies.

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[1] The technique in general which was found to be satisfactory for bee-disease studies is detailed to some extent in the sacbrood paper (17).

[2] The experimental colony is described in earlier papers (17, 18).

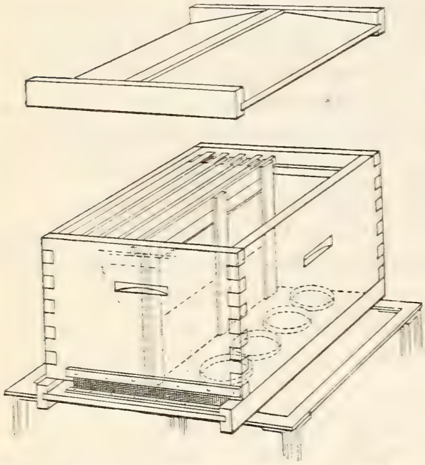

FIG. 6.—Experimental hive, having 4 Hoffman frames, a division board, Petri dishes as feeders, the entrance nearly closed with wire cloth, and the opening on the side of the hive body occupied by the frames. (Author's illustration.)

During most of the time that experimental studies are being made it is necessary to have fresh diseased material at hand. A supply can be maintained by using one or more colonies for this purpose. Repeated inoculations of the colony usually must be made at intervals of a few days or after longer periods, depending on its condition and the need for the virus. The indirect method is especially indicated in inoculating these colonies.

Frequently colonies which have been employed in European foulbrood experiments can be used again for further experiments on the disease. This must be done with some care, however. The condition of the brood always should be noted before an inoculation is made. European foulbrood colonies serve very well the purpose of experimental colonies for the other brood diseases and for Nosema-disease. In fact, not infrequently during these studies experiments on two or more of the diseases were in progress in a colony at the same time.

The apiary (Pl. VI) used in the experimental work with European foulbrood was the same as the one employed in the study of sacbrood (17), Nosema-disease (18), and American foulbrood (19). The hive (fig. 6) and the experimental colonies, where they were not the same, were similar to those used in the other studies. The method of making the inoculations was also similar. The colonies were, therefore, in the open and the bees had free access of flight. The same precautions taken to minimize robbing, swarming, absconding, and drifting of bees were observed in the experiments with this disease as with the other diseases. All hives which had housed European foulbrood colonies were flamed before they were used again to be certain that there would be no infection from such a source. Whether the queens used had been in diseased colonies need not give one any concern. Further reference to the technique followed in the present studies will be made as the experiments are discussed.

THERMAL DEATH POINT OF BACILLUS PLUTON

The result of the experiments recorded by the writer in an earlier paper (16) shows that when suspended in water the thermal death point of *Bacillus pluton* is approximately 63° C., the period of application being 10 minutes. Further experiments have been conducted in which the organism was suspended in honey and heated. After being heated, healthy larvæ are inoculated by feeding, using the direct or pipette method. Table I summarizes the experiments made:

TABLE I.—*Resistance to heat of Bacillus pluton suspended in honey*

Date of inoculation.	Temperature.		Period of heating.	Results of inoculation.
1915.	*°C.*	*°F.*	*Minutes.*	
June 15	67	153	10	European foulbrood produced.
Do	70	158	10	Do.
Do	75	167	10	Do.
June 22	76	169	10	Do.
Do	78	172	10	Do.
Sept. 22	79	174	10	No disease produced.
June 15	80	176	10	Do.
June 18	80	176	10	Do.
Sept. 27	81	178	10	Do.
June 19	85	185	10	Do.
Do	90	194	10	Do.

The results given in the foregoing table show that the thermal death point of *Bacillus pluton* suspended in honey is approximately 79° C., maintained for 10 minutes.

RESISTANCE OF BACILLUS PLUTON TO DRYING

In conducting experiments relative to the effect of drying on *Bacillus pluton* the stomach contents (Pl. VIII) of larvæ sick or recently dead of European foulbrood are spread in a thin layer in Petri dishes or on slides. From time to time after the films are made healthy larvæ are inoculated by feeding a suspension of the drying larval material suspended in a weak sirup solution. When no infection results the germ is considered as having been destroyed. Observations have been made on the virus kept at incubator, room, outdoor, and refrigerator temperatures and shielded from the light in each instance. The experiments conducted with *Bacillus pluton* in these environments are summarized in Tables II, III, IV, and V which follow:

TABLE II.—*Resistance of Bacillus pluton to drying at incubator temperature*

Date of inoculation.	Period of exposure.		Results of inoculation.
	Months.	*Days.*	
1914.			
July 3		10	European foulbrood produced.
July 10		17	Do.
July 17		24	Do.
July 25	1	2	Do.
Aug. 3	1	7	Do.
Aug. 15	1	17	Do.
Sept. 1	2	0	Do.
Sept. 16	2	11	Do.
Sept. 29	3	0	Do.
1915.			
June 29	9	10	Do.
July 13	12	2	Do.
July 9	12	0	No disease produced.
Sept. 20	14	18	Do.
Sept. 17	14	20	Do.
1916.			
Sept. 8	24	0	Do.

TABLE III.—*Resistance of Bacillus pluton to drying at room temperature*

Date of inoculation.	Period of drying.		Results of inoculation.
	Months.	*Days.*	
July 25, 1914	1	1	European foulbrood produced.
Sept. 16, 1914	1	21	Do.
Sept. 1, 1914	2	8	Do.
Sept. 28, 1914	2	21	Do.
Sept. 29, 1914	3	0	Do.
Oct. 6, 1914	3	14	Do.
Jan. 29, 1915	9	10	Do.
July 9, 1915	12	6	Do.
Aug. 3, 1914	11	13	No disease produced.
June 22, 1915	11	15	Do.
June 25, 1915	11	18	Do.
Aug. 9, 1915	12	2	Do.
Sept. 17, 1915	14	10	Do.
Sept. 20, 1915	14	18	Do.
Sept. 8, 1916	24	0	Do.
Do	36	0	Do.

TABLE IV.—*Resistance of Bacillus pluton to drying at outdoor temperature*

Date of inoculation.	Period of drying.		Results of inoculation.
	Months.	*Days.*	
Sept. 2, 1914		33	European foulbrood produced.
Sept. 16, 1914		47	Do.
Oct. 13, 1914		74	Do.
May 26, 1915	9	21	Do.
June 19, 1915	10	18	Do.
May 17, 1915	13	17	Do.
Aug. 3, 1915	12	3	No disease produced.
Aug. 17, 1915	12	17	Do.
June 23, 1916	23		Do.

TABLE V.—*Resistance of Bacillus pluton to drying at refrigerator temperature*

Date of inoculation.	Period of drying.		Results of inoculation.
	Months.	*Days.*	
Oct. 17, 1915	....	26	European foulbrood produced.
Sept. 18, 1916	3	28	Do.
May 3, 1916	6	12	Do.
June 23, 1916	8	0	Do.
May 26, 1916	8	2	Do.
June 23, 1916	9	0	Do.
July 31, 1916	10	7	Do.
Sept. 18, 1916	10	18	Do.

From Table II it will be observed that *Bacillus pluton* in a dry film made from the contents of infected larvæ resisted drying at incubator temperature for approximately one year. Table III shows that at room temperature, other conditions being similar, the resistance is approximately equal to that at incubator temperature. At outdoor temperature, as shown by Table IV, the resistance is again approximately the same. At refrigerator temperature, Table V, the experiments do not include the period at which *Bacillus pluton* is destroyed. In 10 months the organism was still viable and the results of the inoculations indicate from the character of the infection produced after such a period that at refrigerator temperature *Bacillus pluton* will remain alive for a longer period than at the other temperatures studied.

RESISTANCE OF BACILLUS PLUTON WHEN DRY TO DIRECT SUNLIGHT

In experiments relative to the resistance of *Bacillus pluton*, when dry, to the direct rays of the sun, smears are made of the contents of stomachs of European foulbrood larvæ in Petri dishes or on slides, and after becoming dry are exposed to the direct rays of the sun. After intervals reckoned in hours inoculations are made by feeding, using the direct method. Infection resulting from such inoculations shows that the drying has not killed the organism. In Table VI experiments performed in this connection are summarized:

TABLE VI.—*Results of inoculation with Bacillus pluton in a dry film exposed to direct sunlight*

Date of inoculations.	Period of exposure.	Results of inoculations.
	Hours.	
July 21, 1914	3	European foulbrood produced.
Sept. 18, 1913	6	Do.
July 31, 1914	7	Do.
Sept. 22, 1915	8	Do.
July 21, 1914	10	Do.
Sept. 27, 1915	14	Do.
Aug. 7, 1914	15	Do.
July 21, 1914	16	Do.
Aug. 7, 1914	20	Do.
Sept. 10, 1915	20	Do.
Sept. 25, 1915	21	Do.
July 22, 1914	23	Do.
Sept. 8, 1915	27	Do.

TABLE VI.—*Results of inoculation with Bacillus pluton, etc.*—Continued

Date of inoculations.	Period of exposure.	Results of inoculation.
	Hours.	
Sept. 20, 1915	21	No disease produced.
Sept. 24, 1915	23	Do.
Sept. 14, 1915	24	Do.
Aug. 16, 1915	26	Do.
Sept. 14, 1915	26	Do.
Sept. 13, 1915	31	Do.
Sept. 20, 1915	38	Do.
Aug. 3, 1915	40	Do.
Sept. 14, 1915	44	Do.
Aug. 16, 1915	46	Do.
Aug. 23, 1915	63	Do.
Sept. 14, 1915	95	Do.

Observations recorded in Table VI show that *Bacillus pluton* in a dry film made from the contents of the stomachs of larvæ sick or recently dead of European foulbrood resists the direct rays of the sun for from 21 to 31 hours.

RESISTANCE OF BACILLUS PLUTON IN WATER TO DIRECT SUNLIGHT

In performing the experiments relative to the effect of direct sunlight on *Bacillus pluton* suspended in water, an aqueous suspension of the contents of stomachs of infected larvæ is exposed, in a Petri dish with the top removed, to the direct rays of the sun. After intervals reckoned in hours inoculations of healthy larvæ are made to determine whether the organism is viable. The direct method is used. Experiments made in this connection are summarized in Table VII:

TABLE VII.—*Resistance of Bacillus pluton suspended in water exposed to the direct rays of the sun*

Date of inoculation.	Period of exposure.	Results of inoculation.
1915.	*Hours.*	
Aug. 24	1	European foulbrood produced.
Do	2	Do.
Aug. 16	2	Do.
Aug. 8	3	Do.
Aug. 9	3	Do.
Aug. 16	3	Do.
Aug. 24	3	Do.
Sept. 13	4	Do.
Aug. 18	5	Do.
Aug. 16	5	No disease produced.
Sept. 14	5	Do.
Aug. 17	6	Do.
Aug. 20	7	Do.
Sept. 14	7	Do.
Do	8	Do.
Do	9	Do.
July 28	10	Do.
Aug. 20	18	Do.

Table VII shows that *Bacillus pluton*, when suspended in water and exposed to the direct rays of the sun, was destroyed in from 5 to 6 hours.

RESISTANCE OF BACILLUS PLUTON IN HONEY TO DIRECT SUNLIGHT

Experiments were made to determine the resistance of *Bacillus pluton* when suspended in honey to the direct rays of the sun. In these experiments a honey suspension of the organism obtained from the stomachs of infected bees is exposed to the sun in a Petri dish with the top removed. After intervals, reckoned in hours, inoculation tests are made using healthy larvæ and the direct method. Table VIII contains a summary of the experiments performed:

TABLE VIII.—*Resistance of Bacillus pluton suspended in honey and exposed to direct sunlight*

Date of inoculation.	Period of exposure.	Results of inoculation.
1915.	*Hours.*	
Aug. 24	1	European foulbrood produced.
Do	2	Do.
Do	3	Do.
Aug. 3	3	No disease produced.
Aug. 20	3	Do.
Sept. 13	3	Do.
Sept. 19	4	Do.
Aug. 20	5	Do.
Sept. 14	5	Do.
Do	6	Do.
Sept. 11	7	Do.
Sept. 14	8	Do.
Do	9	Do.
Sept. 28	10	Do.

It is shown by the experiments recorded in Table VIII that in direct sunlight *Bacillus pluton* was destroyed in from 3 to 4 hours.

The results obtained by the experiments summarized in the last three tables above, it will be noted, show that *Bacillus pluton* is susceptible to the destructive effects of the direct rays of the sun; that the resistance of the organism suspended in honey is about equal to its resistance when suspended in water; and when dry the resistance is considerably greater than when suspended in either water or honey. It is to be expected that the period required for the destruction of the organism by the rays of the sun will vary with the intensity of the rays at the time of the exposure. In the foregoing experiments clear days were chosen and preference was given to the middle of the day for the exposures.

RESISTANCE OF BACILLUS PLUTON TO FERMENTATION

In obtaining data relative to the resistance of *Bacillus pluton* to fermentation, the stomach contents of larvæ sick or recently dead of European foulbrood were suspended in a 10 per cent sugar (saccharose) solution. A bit of soil was added to inoculate it further. Records were made on suspensions fermenting at incubator and room temperatures, respectively. Tables IX and X which follow summarize experiments made:

TABLE IX.—*Bacillus pluton in a 10 per cent sugar solution fermenting at incubator temperature*

Date of inoculation.	Period of fermentation.	Results of inoculation.
	Days.	
Aug. 12, 1916	3	European foulbrood produced.
June 26, 1916	5	No disease produced.
Sept. 2, 1916	7	Do.
Sept. 7, 1915	8	Do.
Aug. 9, 1915	10	Do.
June 30, 1916	15	Do.
July 5, 1915	15	Do.
Aug. 24, 1915	24	Do.

TABLE X.—*Bacillus pluton in a 10 per cent sugar solution fermenting at room temperature*

Date of inoculation.	Period of fermentation.	Results of inoculation.
	Days.	
June 30, 1916	9	European foulbrood produced.
July 17, 1915	10	Do.
Sept. 8, 1915	10	Do.
July 21, 1915	14	Do.
Aug. 25, 1916	16	Do.
July 8, 1916	17	Do.
Sept. 10, 1916	11	No disease produced.
July 5, 1916	14	Do.
Aug. 26, 1916	21	Do.
Aug. 3, 1915	27	Do.
Aug. 9, 1915	32	Do.
Aug. 25, 1915	49	Do.

The experimental results contained in Tables IX and X show that *Bacillus pluton* is destroyed in a fermenting solution. At incubator temperature the virus was destroyed in from 3 to 5 days, and at room temperature it was killed in from 11 to 21 days.

Similar experiments were made in which suspensions in 20 per cent honey solutions were allowed to ferment at outdoor temperature. The records obtained show that *Bacillus pluton* in this environment was still alive and virulent after one month.

RESISTANCE OF BACILLUS PLUTON TO PUTREFACTION

Suspensions of the contents of stomachs from larvæ sick or dead of European foulbrood were made in a 1 per cent peptone solution. Soil was added to inoculate it further. Putrefactive changes were allowed to take place at incubator and room temperatures, respectively. In Tables XI and XII. which follow, are summarized the experiments performed:

TABLE XI.—*Bacillus pluton in the presence of putrefactive processes at incubator temperature*

Date of inoculation.	Period of putrefaction.	Results of inoculation.
	Days.	
June 30, 1916	9	European foulbrood produced.
Sept. 2, 1916	7	No disease produced.
Aug. 15, 1916	8	Do.
Sept. 7, 1915	13	Do.
July 5, 1916	15	Do.
Sept. 10, 1915	16	Do.
July 8, 1916	18	Do.
Aug. 23, 1915	19	Do.
Aug. 30, 1915	26	Do.

TABLE XII.—*Bacillus pluton in the presence of putrefactive processes at room temperature*

Date of inoculation.	Period of putrefaction.	Results of inoculation.
	Days.	
Aug. 4, 1914	8	European foulbrood produced.
July 17, 1915	10	Do.
July 5, 1916	14	Do.
July 23, 1915	16	Do.
Aug. 14, 1914	18	Do.
Sept. 17, 1915	18	Do.
Aug. 25, 1916	18	Do.
Sept. 2, 1916	26	Do.
Aug. 3, 1915	27	Do.
Aug. 28, 1916	21	No disease produced.
Sept. 1, 1914	35	Do.
Sept. 16, 1914	51	Do.
Aug. 12, 1916	52	Do.

As shown by Tables XI and XII *Bacillus pluton* is destroyed in the presence of putrefactive processes. At incubator temperature it resisted the effects of these processes for from 7 to 13 days and at room temperature for from 21 to 35 days.

During August and September, 1916, preliminary experiments were made testing the resistance of *Bacillus pluton* to putrefaction at outdoor temperature. The parasite was alive and virulent after 40 days. The maximum period during which it will remain so has not been determined.

VIABILITY OF BACILLUS PLUTON IN HONEY

Honey suspensions of *Bacillus pluton* from the stomach contents of larvæ sick or recently dead of European foulbrood were made and distributed in flasks each containing about 300 c. c. These were allowed to stand at room temperature shielded from the light. At intervals thereafter colonies free from the disease were inoculated each with the contents of a single flask. A summary of the experiments is contained in Table XIII:

TABLE XIII.—*Resistance of Bacillus pluton in honey at room temperature*

Date of inoculation.	Period in honey.		Results of inoculation.
	Months.	*Days.*	
May 22, 1915		4	European foulbrood produced.
June 12, 1915		25	Do.
July 23, 1915	1	6	Do.
June 25, 1915	1	11	Do.
Aug. 23, 1915	1	15	Do.
Aug. 3, 1915	1	17	Do.
July 12, 1915	1	25	Do.
Aug. 23, 1915	2	7	Do.
Sept. 10, 1915	2	25	Do.
Aug. 16, 1916	3	0	Do.
May 19, 1916	7	0	No disease produced.
May 4, 1915	7	17	Do.
June 7, 1913	8	0	Do.
June 13, 1913	8	0	Do.
May 13, 1915	8	25	Do.
May 14, 1915	8	26	Do.
May 22, 1915	9	5	Do.
May 24, 1915	9	5	Do.
July 31, 1916	9	11	Do.
May 15, 1916	10	6	Do.

Experimental evidence recorded in Table XIII shows that the virus of European foulbrood when suspended in honey at room temperature ceased to be virulent in from 3 to 7 months.

VIABILITY OF BACILLUS PLUTON IN POLLEN

Preliminary experiments were made to determine the viability of *Bacillus pluton* in pollen. Pollen is removed from brood-comb, and an aqueous suspension of the organism obtained from the stomachs of larvæ sick or recently dead of the disease is added to it until a moderately thick, pastelike mass is obtained. This is distributed in Petri dishes and allowed to stand at room and refrigerator temperatures, respectively. After different intervals of time the contents of a single dish, after being suspended in water, are added to about 300 c. c. of sirup and the suspension is fed to a colony, using the indirect method. The results show that *Bacillus pluton* was virulent after 7 months at room temperature and for more than 10 months in the refrigerator. The maximum period during which the organism will remain alive in these two environments has not been determined.

RESISTANCE OF BACILLUS PLUTON TO CARBOLIC ACID

Preliminary experiments were made to determine the effect of carbolic acid on the virus of European foulbrood. An aqueous suspension of the contents of the stomachs of larvæ sick or dead of the disease is first made. A measured quantity of this suspension is added to an equal quantity of an aqueous suspension of carbolic acid of a strength twice that desired in the experiment. After shaking, it is allowed to stand at room temperature. At intervals brood free from the disease is fed a bit of this suspension, using the direct method. Table XIV summarizes the experiments performed:

TABLE XIV.—*Effect of carbolic acid on Bacillus pluton*

Date of inoculation.	Strength of solution.	Period of suspension.	Results of inoculation.
	Per cent.	*Days.*	
Aug. 22, 1914	½	¾	European foulbrood produced.
Aug. 14, 1914	½	1	Do.
July 3, 1915	½	4	Do.
Aug. 21, 1914	½	8	Do.
Sept. 4, 1914	½	18	No disease produced.
June 29, 1915	1	[1]5	European foulbrood produced.
July 3, 1915	1	4	No disease produced.
June 29, 1915	2	[1]5	Do.
Aug. 22, 1914	2	[1]18	Do.
Aug. 14, 1914	2	1	Do.
Aug. 17, 1914	2	4	Do.
Aug. 25, 1914	2	4	Do.
Aug. 22, 1914	2	9	Do.
June 29, 1915	4	[1]5½	Do.

[1] Hours.

The experiments outlined in Table XIV show that *Bacillus pluton* withstood a one-half per cent solution of carbolic acid for 8 days but not for 18 days; that it withstood 1 per cent for 5 hours but not for 4 days; and that it was destroyed by 2 and 4 per cent solutions, respectively, in less than 6 hours. Probably it is destroyed by these latter strengths in considerably less time than this.

It is seen by these preliminary experiments that *Bacillus pluton* is destroyed easily by carbolic acid as a disinfectant. As a drug, however, less can be expected of it, inasmuch as a strength twice that which the bees will accept in honey (Table XV) requires days to destroy the germ. While the fact does not furnish conclusive proof of the value of carbolic acid as a drug, it indicates what might be expected of it in the treatment of the disease.

In using the results recorded on the foregoing pages for the purpose of destroying the virus of European foulbrood and controlling the disease in practical apiculture, it must be borne in mind, as has been urged in the discussions on the other bee diseases, that due allowance must be made by the beekeeper for variations which always occur. These, however, are relatively slight and can be met readily. In the destruction of the virus through heating, for example, the temperature can be raised a few degrees above that which is found to be the minimum required, or the time can be extended somewhat. Similarly for the other destructive agencies the effectiveness of the process can be increased.

EFFECT OF DRUGS ON EUROPEAN FOULBROOD

Preliminary experiments have been made to obtain data relative to the effect of drugs on *Bacillus pluton.* In conducting the experiments a suspension of the stomach contents of larvæ sick or recently dead of European foulbrood is made in an aqueous solution of the drug. This is added to diluted honey and healthy brood is fed this suspension. In some instances the direct and in others the indirect method was followed. In Table XV are summarized the experiments which were performed:

Table XV.—*The effect of drugs on European foulbrood*

Date of experiment.	Drugs.	Strength.	Results of inoculation.
1916.			
July 11	Betanaphthol	1:2000	European foulbrood produced.
May 31	do	1:1000	
June 7	do	2:1000	
July 11	Carbolic acid	1:2000	
June 21	do	1:1000	
Do	do	2:1000	
May 31	Oil of eucalyptus	4:1000	
July 11	do	4:1000	
June 7	Formic acid	1:1000	
July 11	do	3:1000	
Do	Salicylic acid	1:2000	
May 31	do	1:1000	
June 7	do	2:1000	
July 11	Salol	1:2000	
May 31	do	1:1000	
June 7	do	2:1000	
July 11	Quinin	2:1000	
May 31	do	4:1000	
June 7	do	10:1000	

It will be observed from Table XV that European foulbrood was produced in all cases in which larvæ were fed a suspension of *Bacillus pluton* in sirup medicated with betanaphthol, carbolic acid, eucalyptus, formic acid, salicylic acid, salol, and quinin (bisulphate of quinin), respectively, in the proportions noted.

The strongest solutions of the drugs used in the experiments are in most instances approximately the maximum proportion of the chemical in honey that will be taken by the bees. These preliminary results indicate that drugs should not be depended upon, for the present at least, in the treatment of European foulbrood, and emphasize the fact that beekeepers should make sure that the value of a drug has been demonstrated fully before it is used.

TRANSMISSION OF EUROPEAN FOULBROOD

While there is yet much to be learned concerning the transmission of European foulbrood, the data at hand relative to this important phase in the study of the disease justify certain statements in regard to it. The disease can be produced experimentally by feeding a healthy colony the crushed larvæ sick or dead of the disease, suggesting that infection takes place by way of the alimentary tract.

Through the study of microtome sections of such larvæ, it has been conclusively proved that infection takes place in this way. The fact is naturally one of special moment in the solution of the transmission of the disease. There is a tendency on the part of adult bees to remove sick and dead larvæ from the brood comb. This is done largely at least in a piecemeal manner. Were the fate of the fragments removed known definitely the solution of the problem naturally would be aided greatly.

If infective material thus removed were fed to susceptible healthy larvae, disease would result. On the other hand should the fragments of diseased larvæ be stored with the honey of the hive or with the pollen, or consumed by the adult bees, or by larvæ later in the feeding stage, the chances that such material would ever reach susceptible larvæ to cause infection are very much reduced. Stored in honey the virus remains virulent only a few months (p. 24); in pollen, however, it remains virulent much longer (p. 24). Drying within the hive *Bacillus pluton* would probably remain alive more than a year (p. 19).

The chances that any portion of the infectious material of any given fragment, if it is removed entirely from the hive by the bees of the colony, and released from them, will be taken up by other bees and carried to healthy brood and cause infection are comparatively slight. If thus removed and exposed to the direct rays of the sun, the virus will be destroyed within a few hours (p. 19); or if subjected to fermentative or putrefactive processes it will be destroyed in a few weeks (p. 23). If *Bacillus pluton* is present in honey extracted from diseased colonies it will be destroyed within a few months while in storage (p. 24). It is seen, therefore, that in nature there are many means that destroy the virus of European foulbrood and thus limit the spread of the disease.

All of the colonies of the experimental apiary used in making the inoculations cited in the present paper had free access to the fields and there was no evidence at any time of the transmission of the disease from infected to healthy colonies. This fact supports the conclusion that the disease is not spread by way of flowers visited by bees from healthy colonies which had been visited previously by bees from diseased ones. The fact further indicates that if the disease is transmitted at all by way of the water supply of the bees, it takes place to a limited extent only. The fact still further indicates that if drones or straying or drifting workers transmit European foulbrood they do so to a slight extent only. If these observations are at variance with the experience of the practical beekeepers, as the writer has been informed that they are, they will probably be of particular interest.

Observations made during the present studies indicate that queens from European foulbrood colonies are not likely to transmit the disease when introduced into healthy colonies. The experiences further show, and the facts in general regarding the disease support the conclusions, that the infection will not be transmitted by the hands or clothing of the beekeeper, or by visitors to the apiary when the manipulations ordinarily practiced are followed. Tools and equipment used about the apiary are not to be feared unless they supply a source for robbing. Hives which have housed infected colonies are not likely to be a medium for the spread of the disease.

Robbing of infected colonies is the most fruitful source of infection. A colony weakened by disease (p. 5) becomes a prey for other bees. Infectious material is carried to other colonies, thereby transmitting the infection. Manipulations in the apiary, whereby brood combs from diseased colonies are placed in healthy ones, are another fruitful source for the transmission of the disease. Preliminary work[1] indicates that stored brood combs from European foulbrood colonies may transmit the disease after a considerable period.

The disease, it would seem, might be spread through the medium of honey from infected colonies. The danger from this source, however, probably has been overestimated at times (p. 23). That pollen stored in the comb would serve as a protection to *Bacillus pluton*, if the parasite were lodged with it, has been determined (p. 24).

DIAGNOSIS

The diagnosis of European foulbrood offers more difficulty than does that of either American foulbrood or sacbrood. It can usually be made, however, from the symptoms alone. Inasmuch as these symptoms (p. 4) are rather varied, much care should be exercised in diagnosing the disease.

The appearance of the adult bees does not aid in the diagnosis. A weak colony should arouse suspicion. Increased suspicion is justified when no other readily discernible cause for the weakness is to be observed. The disease may be present, however, in a strong colony. Such a case may be one of recent infection or one which late in the recovery from the disease has gained in strength. It may be, however, a colony which has suffered only a slight attack of the disease.

The following outstanding gross characters are often sufficient for a diagnosis: The dying of the brood before the time for capping (Pls.

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[1] Brood combs were removed from European foulbrood colonies in October, 1914, and stored in the laboratory. In May, 1915, one frame of brood comb was placed in each of two colonies with the result that European foulbrood was produced in both instances. When a frame of the comb was placed in the colony in May, 1916, no disease resulted. After 6 months the combs were still able to transmit the disease; after 18 months they did not. These experiments are not sufficient to justify definite conclusions but are suggestive.

II, III, IV), the yellow hue of the larvæ more recently dead, and the brown shade of those longer dead, the irregularity of the brood (Pl. I), and the absence of a disagreeable odor.

Not infrequently, however, the diagnosis is not so simple. During recovery from the disease scales (Pl. V, F, I) of larvæ dying in capped cells may be the only remains of diseased brood to be found, all of the younger larvæ having been removed by the bees. These scales[1] are, as a rule, comparatively few in number and resemble somwhat those of American foulbrood, but would rarely be mistaken for those of sacbrood. In these cases a diagnosis can be made frequently by a microscopic examination alone. Cultures, however, are needed in some instances.

Special attention is needed in cases of early infection and in other instances where only a small amount of diseased brood in uncapped cells is present (Pl. I, A). The symptoms manifested by larvæ sick or only recently dead of the disease furnish often the readiest and most conclusive evidence of the presence of the disease. Larvæ of the age at which they comfortably fill the bottom of the cell exhibiting increased peristalsis-like movements of the body suggest European foulbrood. Increased transparency of larvæ of this age (Pl. II, B) is also suggestive. The presence of a white or yellowish-white mass within the stomach (midgut) as seen through the dorsal median line of the body is strong evidence of the presence of the disease. If on puncturing the body of larvæ nearly dead or only recently dead the contents of the stomach flows out as a fluid and more or less finely granular mass, the fact furnishes further evidence of European foulbrood.

A symptom which is pathognomonic of the disease is to be seen in larvæ that have been infected somewhat more than two days, but wherein the disease has not reached an advanced stage. The test (15) involves the removal of the stomach contents, which consist of a bacterial mass, together with a small amount of larval food and a clear envelope (Pl. VIII, a, b, c). The slight tension necessary to remove the contents stretches the envelope and breaks the whitish bacterial mass into a number of fragments.

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[1] The number of larvæ that die of European foulbrood in capped cells after assuming the endwise position represents a very small percentage of the brood that dies of the disease. These remains may be found in practically all colonies in which the disease has been present for a sufficiently long period and in which a considerable amount of dead brood has resulted. Before becoming dry they are somewhat viscid and are less easily removed than are those of larvæ dying at an earlier age. These and the scales resulting from them are used in diagnosis principally (1) when the younger larvæ sick or dead of the disease have been removed, (2) when a demonstration of the presence of *Bacillus alvei* is desired, and (3) when both European foulbrood and American foulbrood infection is suspected. Such a double infection has been encountered in the writer's experience very rarely. In making diagnoses, therefore, after European foulbrood has been found in the sample American foulbrood is seldom looked for.

By one or more of these colony symptoms manifested by larvæ sick or only recently dead of the disease the experienced can diagnose European foulbrood definitely without a microscopic examination. The methods not only give definite results, but are also easy of application. They have been indispensable in much of the writer's experimental work and it is believed that the beekeeper will find them to be valuable in practical apiculture where other gross methods fail.

BACTERIOLOGICAL EXAMINATION

The findings from microscopic examinations and from cultures have been set forth in an earlier publication (10). These are always adequate for a definite diagnosis when a suitable sample is at hand. *Bacillus alvei* (p. 11) (fig. 2; Pl. VII, D, F) frequently overshadows all other species. In larvæ sick of the disease *Bacillus pluton* (Pl. VII, A, B) overshadows all others. With experience one learns to recognize this species in stained preparations. The individuals are seen frequently in groups. They are more or less lancet shaped, and a variation in size is often sufficient to be noticeable (fig. 1).[1] In larvæ nearly dead and in those only recently dead *Bacterium eurydice* (p. 13) (fig. 4; Pl. VII, C) is frequently encountered. *Streptococcus apis* (p. 12) (fig. 3; Pl. VII, E) occurs in a small number of cases. *Bacillus orpheus* (p. 14) (fig. 5; Pl. VII, H), *B. vulgatus*, and *B. mesentericus* are occasionally encountered. While *B. pluton* is present in all cases of European foulbrood, not infrequently in routine examinations it is so masked by the secondary invaders that the microscopic examination fails to reveal it. In many cases *B. alvei* and *B. orpheus* are recognized microscopically. Cultures are necessary for the differentiation of *B. vulgatus* and *B. mesentericus*. In many cases cultures are needed to differentiate *Strep. apis* and *B. pluton*. *Strep. apis* grows on the ordinary media, *B. pluton* does not.

DIFFERENTIAL DIAGNOSIS

AMERICAN FOULBROOD

American foulbrood is recognized by the death of larvæ in capped cells and of pupæ soon after transformation, the viscidity of the decaying remains of the brood, and the "foulbrood" odor which is frequently present. The presence of the spores of *Bacillus larvae* in large numbers and the absence of other species is conclusive proof of American foulbrood.

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[1] Smears made from larvæ sick of European foulbrood and quite early in the course of the disease were selected in making a study of the morphology of *B. pluton*. These were stained with iron hematoxylin. In smears made from dead larvæ and stained with carbol fuchsin, as is usually done, the pointed ends and the more or less rod-shaped forms are less prominent than illustrated in figure 1.

SACBROOD

Sacbrood is recognized by the death of larvæ after capping, by the saclike appearance, the watery granular consistency of the larval remains, and the absence of viscidity. The absence of microorganisms characterizes the microscopic picture in sacbrood.

OTHER CONDITIONS

Conditions referred to as chilled brood, overheated brood, and starved brood must be differentiated from European foulbrood. This can usually be done with little difficulty by a comparison of the symptoms present with those of European foulbrood. The history of the case is of much value. Brood dying after being removed from the hive and before examination is made shows often an interesting similarity to European foulbrood. *B. alvei* and *B. pluton* are not found in these conditions. The absence of bacteria, or their presence in small numbers only, and a lack of uniformity of the species when present, characterize the bacteriological findings in these cases.

PROGNOSIS

There is no uniformity in the prognosis in European foulbrood. The diseased colony may recover completely from the infection, suffering only a slight loss in strength as a result of it; the colony may recover but sustain considerable loss; or it may die out entirely, as a result of the disease. The infection may spread only slightly to other colonies of the apiary or the entire apiary may become infected. The losses sustained vary from slight to total. The tendency for European foulbrood to disappear is greater after midsummer than before.

Whether a larva once infected ever recovers from this disease is not known, but the evidence at hand indicates that it may. This seems to be especially probable when the infection takes place during the latter part of the feeding period of the larva. Queen larvæ are susceptible to infection, but sufficient data are wanting from which to estimate the extent to which queenlessness may result from the disease. In experimental colonies queens have been reared in the presence of a considerable amount of European foulbrood infection.

The prognosis for the colony in the case of European foulbrood may be said, therefore, to vary from very good to very grave, many recovering entirely from the infection without treatment and without appreciable losses, while others rapidly decline and finally die out.

SUMMARY AND CONCLUSIONS

The following is a brief summary of facts regarding European foulbrood, together with some conclusions based upon them:

1. European foulbrood is an infectious brood disease of bees caused by *Bacillus pluton.*

2. All larvæ—worker, drone, and queen—are susceptible to the disease; adult bees are not.
3. Man evidently is not susceptible to infection with *Bacillus pluton* nor are the experimental animals.
4. As far as is known insects other than bees are not susceptible.
5. Brood can be infected by feeding the colony a suspension of crushed larvæ sick or dead of the disease. This is described in the present paper as the indirect method.
6. The virus contained in a single larva recently dead of European foulbrood will produce a considerable amount of disease when fed to a colony.
7. The larvæ can be infected also by a more direct method. A fraction of a drop of a suspension of the stomach contents of a larva sick of the disease added with a capillary pipette directly to the food surrounding the larva to be inoculated will result in infection.
8. *Bacillus pluton* gains entrance to the larva by way of the mouth. The growth and multiplication of the parasite take place within the stomach (mid-intestine) of the larva and do not, during the life of the larva, get beyond the peritrophic membrane. The tissues, therefore, are not invaded by it.
9. The secondary invaders in European foulbrood, *Bacillus alvei*, *Streptococcus apis*, *Bacterium eurydice*, and *Bacillus orpheus*, rarely, if ever, invade the tissues until the larva is dead or nearly so. In a few instances in microtome sections rod forms have been encountered in the act of invading the tissues of living larvæ. The species, however, was not determined definitely.
10. The period of incubation is slightly less than 3 days.
11. Brood is susceptible to infection at all seasons of the year.
12. More brood die of the disease during the first half of the brood-rearing season than during the second half.
13. The writer has examined samples of the disease from Canada and the United States. From written reports it seems quite certain that it occurs also at least in Denmark, England, Germany, France, and Switzerland.
14. Occurring as it does in this somewhat wide range of climatic conditions, the presence of the disease in any particular locality can not be attributed entirely to the prevailing climatic conditions.
15. The quality of food obtained by the bees does not affect greatly, if at all, the course of the disease in the colony, although the quantity may affect it to a variable extent.

16. Experimental colonies may be inoculated and kept in the apiary without transmitting the disease to others. This fact is of special importance, not only in connection with the technique of making studies on the disease, but also in the control of the malady.
17. The thermal death point of *Bacillus pluton* suspended in water is approximately 63° C. maintained for 10 minutes.
18. When suspended in honey *Bacillus pluton* is destroyed in 10 minutes at approximately 79° C.
19. Drying at room or incubator temperature *Bacillus pluton* remains alive and virulent for approximately one year.
20. When dry, *Bacillus pluton* resisted the direct rays of the sun for from 21 to 31 hours.
21. When suspended in water *Bacillus pluton* was destroyed by the direct rays of the sun in from 5 to 6 hours.
22. When suspended in honey and exposed to the direct rays of the sun *Bacillus pluton* was destroyed in from 3 to 4 hours.
23. In the presence of fermentative processes in a 10 per cent sugar solution *Bacillus pluton* was destroyed in from 3 to 5 days at incubator temperature and in from 11 to 21 days at room temperature.
24. In a fermenting honey solution outdoors *Bacillus pluton* was still alive and virulent after one month.
25. In the presence of putrefactive processes at incubator temperature *Bacillus pluton* was destroyed in from 7 to 13 days and at room temperature in from 21 to 35 days.
26. In a putrefying medium at outdoor temperature *Bacillus pluton* remained alive and virulent for more than 40 days. The maximum period has not been determined.
27. In honey at room temperature *Bacillus pluton* ceased to be virulent in from 3 to 7 months.
28. Mixed with pollen, *Bacillus pluton* remained alive and virulent for more than 7 months at room temperature and more than 10 months at refrigerator temperature, the maximum time not being determined.
29. In one-half per cent carbolic acid solution *Bacillus pluton* was destroyed in from 8 to 18 days; in 1 per cent it was destroyed in from 5 hours to 4 days, and in 2 and 4 per cent in less than 6 hours. The probability is that at these higher strengths of the solution minutes rather than hours are sufficient for the destruction of the virus.
30. Experimental evidence indicates that at the present time drugs should not be depended upon in the treatment of European foulbrood.

31. Robbing from diseased colonies of the apiary or from neighboring apiaries is the most likely manner in which European foulbrood is transmitted in nature.
32. Brood-combs containing diseased brood, if given to a healthy colony, serve as a medium for the transmission of the disease.
33. European foulbrood is not likely to be transmitted by queens or drones. Whether they ever do so has not been demonstrated.
34. As a rule a hive which has housed a European foulbrood colony should not be considered as a fruitful source of infection. The facts indicate that often such hives could be used with impunity for housing colonies without treatment. Flaming them inside certainly removes all danger.
35. The transmission of European foulbrood by way of flowers, visited by bees from diseased colonies and subsequently by those from healthy ones, is not to be considered as a likely source of infection. Whether the water supply is ever a source of danger is not known. It is evidently not a fruitful source.
36. The disease is not likely to be transmitted through the medium of the clothing or hands of the apiarist.
37. Tools and bee supplies in general do not serve as means for the transmission of the disease in the absence of robbing from such sources.
38. It is usually possible to diagnose European foulbrood from the symptoms alone. A definite diagnosis can be made from suitable samples by bacteriological methods.
39. The prognosis in European foulbrood varies from very good to exceedingly grave. The tendency for a colony to recover entirely from the disease is much greater than in American foulbrood.
40. Considered from the technical point of view, much is yet to be learned concerning European foulbrood. For practical purposes, however, it can be said that sufficient knowledge has been gained to make it possible for the beekeeper to devise a treatment which will be logical, efficient, and at the same time economical.

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EXPLANATION OF PLATES

PLATE I

Brood-combs containing larvæ that are sick and others that are dead of European foulbrood, showing the irregular appearance of the brood. About one-half natural size.

A.—The dead larvæ have all been removed. Some of the remaining larvæ are sick, others are not infected. The disease was produced by experimental inoculation.

B.—Many of the dead larvæ have not been removed. The comb had been out of the colony for a considerable period. The larvæ that are quite young showing abnormal position and appearance are not sick or dead of European foulbrood, but are so as a result of the comb being away from the colony. Disease was produced by experimental inoculation.

C.—The comb was taken from a colony in which the disease had appeared in nature and not as the result of artificial inoculation. Before being photographed the brood-comb had been out of the hive for a few days. Aside from the larvæ which are dead of European foulbrood, other larvæ present are dead from lack of attention by adult bees—starvation, exposure, and other causes.

PLATE II

A.—Live larva showing first symptoms of European foulbrood. The turgidity is slightly less than in a healthy larva (D).

B.—Live larva showing early symptoms of European foulbrood. The body is more transparent than that of a healthy larva (D). Small opaque areas give it a punctate appearance.

C.—Larva dead of European foulbrood contained within a chitinous envelope filled with a watery-appearing fluid.

D.—Healthy larva of the earliest age at which larvæ die of European foulbrood. Turgidity marked.

E.—European foulbrood larva which may or may not be dead. Surface less glistening than in healthy larvæ. Marked turgidity lost. Prominence of tracheæ not increased.

F.—Scale formed by drying of larvæ dead at early age. Prominence of tracheæ marked.

G.—View of healthy larva in normal position with roof of cell removed. Larva turgid. Surface glistening.

H.—Larva sick with European foulbrood. Lack of turgidity and increased prominence of tracheæ observed.

I.—European foulbrood larva which may or may not be dead. Less turgidity, a relative dullness in the surface appearance, and punctate condition present. Similar to E.

PLATE III

A.—Healthy larva immediately preceding the age at which the capping of the cell is done. Dorsal surface turned toward the observer. The narrow transparent area along the dorsal median line is prominent.

B.—Larva dead of European foulbrood of the same age as A. The turgidity, glistening surface, and transparent area are less marked.

C.—Larva dead of European foulbrood partly coiled and partly endwise in cell.

D.—Healthy larva near the age at which capping takes place.

E.—Dorsolateral view of a larva dead of European foulbrood. The ends are directed toward the bottom of the cell.

F.—Larva dead of European foulbrood. The body occupies a spiral position in the cell.

G.—Healthy larva approaching the age at which capping takes place.

H.—Lateral view of larva dead of European foulbrood seen with the roof of the cell removed. The ends are directed toward the bottom and the dorsal surface toward the mouth of the cell.

I.—Dead larva similar to H but having been dead somewhat longer.

PLATE IV

A.—Young larva dead of European foulbrood. The chitinous capsule and tracheæ are prominent.

B.—Fragments of young larva dead of European foulbrood, a portion having been removed by adult bees after its death.

C.—Lateral view of larva dead of European foulbrood, the roof of the cell having been removed. The ends in this instance are directed more or less toward the mouth of the cell.

D.—Lateroventral view of larva dead of European foulbrood. The body lies with the dorsal portion against the floor of the cell.

E.—Larva dead of European foulbrood lying on the floor of the cell in somewhat lengthwise position.

F.—Scale of European foulbrood larva which had occupied a somewhat spiral position in the cell.

G.—Scale of a European foulbrood larva which had occupied a position somewhat as shown in D. This scale and the one shown in F can be removed intact rather easily and without tearing the wall of the cell.

PLATE V

Larvæ (prepupæ) of bees dead of European foulbrood which had already assumed before death a lengthwise position in the cell.

A.—Fragment of European foulbrood soon after death. A portion of the larva has been removed by the adult bee.

B.—Entire cap of cell containing larva dead of European foulbrood.

C.—Punctured cap of cell containing the remains of a larva dead of European foulbrood.

D.—End view of larva dead of European foulbrood.

E.—End view of larva dead of European foulbrood, lying with its dorsal surface against the floor of the cell. Considerable drying of the remains has taken place.

F.—End view of scale of European foulbrood larva which had reached before death the age at which the endwise position in the cell is assumed.

G.—Ventral view of European foulbrood larva. Stage similar to D. Turgidity is lost to a large extent and the segmented markings are less distinct than in healthy larvæ.

H.—Larva which has been dead of European foulbrood for a longer period than illustrated in G. The ridge and furrows indicating the segments of the body are not marked.

I.—Scale of European foulbrood similar to F. The larva before death had reached the endwise position in the cell. These scales resemble very much those of American foulbrood. They are more easily removed, however, do not adhere so closely to the floor of the cell, and are more rubberlike in consistency, breaking less readily than those of American foulbrood.

PLATE VI

A view of the experimental apiary of 54 colonies in which the inoculation experiments made during the summer of 1915 were conducted.

PLATE VII

Photomicrographs illustrating the more commonly encountered bacteria in European foulbrood.

A.—*Bacillus pluton:* A smear from the stomach of a larva sick with European foulbrood. Note the paired forms and short chains. These forms are numerous in a recent infection, suggesting the organism in the process of multiplication. The lancet-shaped form is by far the predominant one in all later stages of the disease. × 1000.

B.—*Bacillus pluton:* A smear from a larva quite recently infected. The multiplying paired forms are at this stage present almost exclusively. × 1000.

C.—*Bacterium eurydice:* Stained preparation from a pure culture on the surface of agar. × 1000.

D.—*Bacillus alvei:* Stained preparation showing spores and spore formation. × 800.

E.—*Streptococcus apis:* Stained preparation from a pure culture. × 800.

F.—*Bacillus alvei:* The peculiar arrangement of the spores as sometimes seen. From a pure culture, the smear having been made by suspending the culture on the slide in normal salt solution. × 1000.

G.—*Bacillus orpheus:* Stained preparation made from a pure culture only a few hours old. Grown on the surface of agar. × 1000.

H.—*Bacillus orpheus:* Stained preparation showing spore formation. Note the stained portion along one side and about both ends of the spore. The stage is soon reached in a culture at incubator temperature. At room temperature it remains in this stage for a considerable period. × 800.

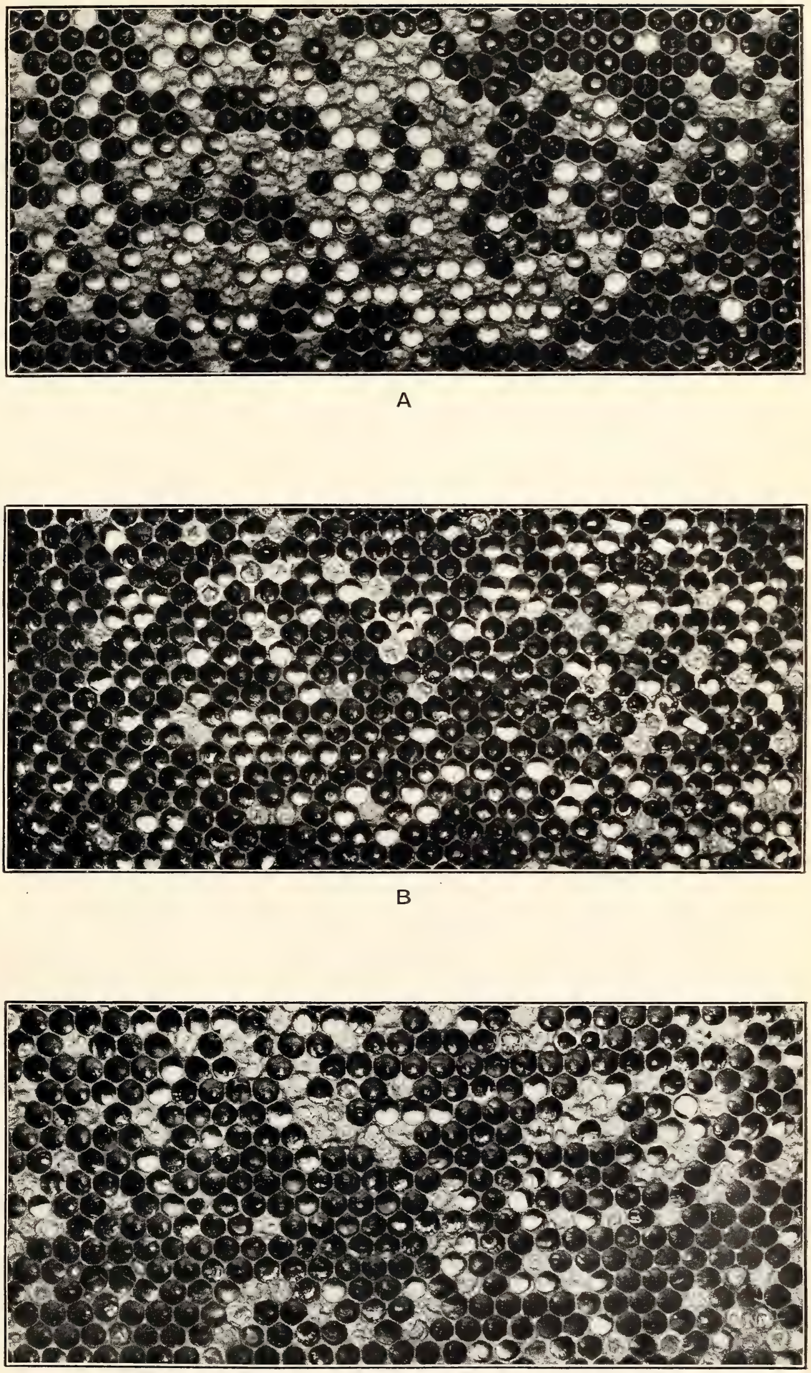
I.—Longisection of a young larva showing early infection in European foulbrood. The bacterial growth is seen as a narrow black area just within the peritrophic membrane on one side of the food mass.

J.—Longisection of larva sick of European foulbrood, showing a later stage of infection than that present in I. The dark area in the food mass shows the bacterial growth. Note that the growth mass does not extend beyond the peritrophic membrane and that it does not extend uniformly along this membrane and throughout the food mass.

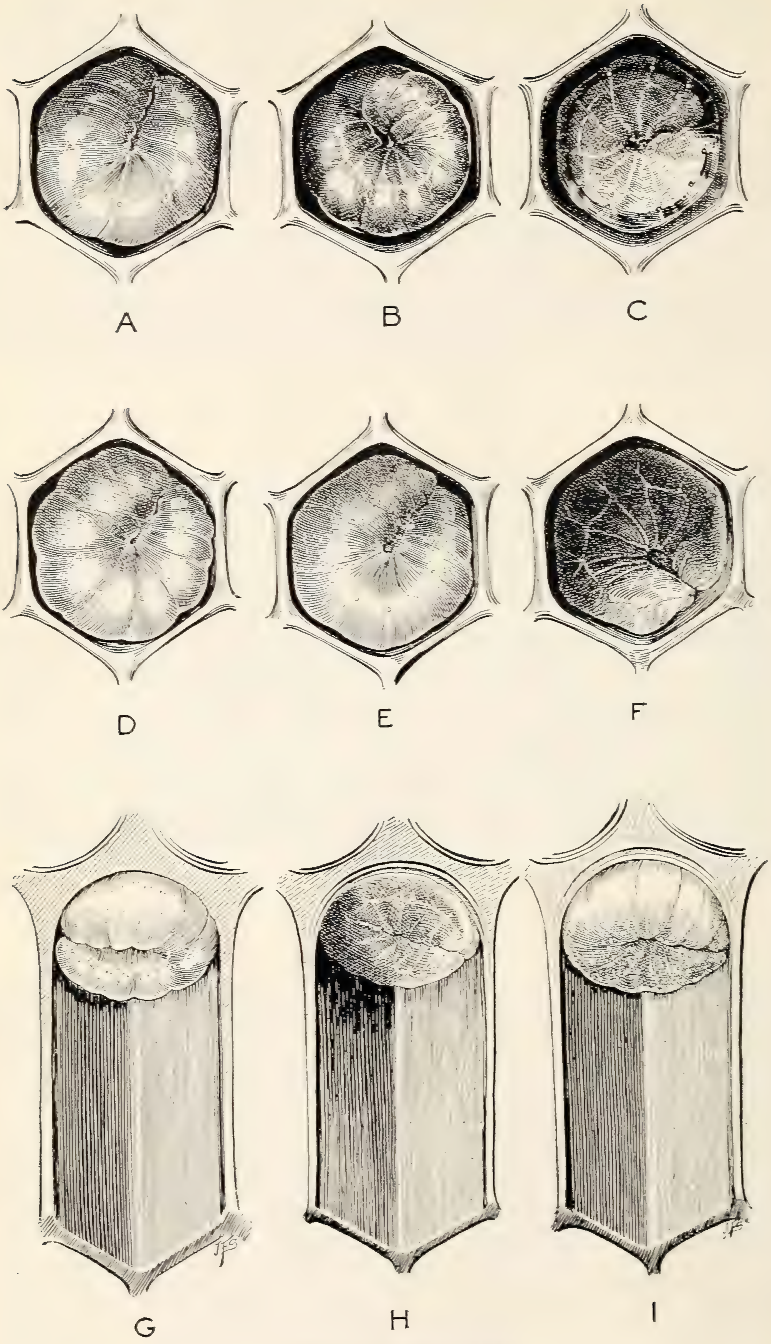
K.—Transverse section of larva about the time of its death from European foulbrood infection. Note the bacterial mass along the peritrophic membrane and extending from the membrane into the food mass. As seen within the living larva this bacterial mass in the sick larva is practically white, but is more or less yellowish white when present with larval food material. The gelatinous-like envelope outside the peritrophic membrane and inside the stomach epithelium in healthy larvæ thins out as the disease advances.

PLATE VIII

The stomach contents of larvæ sick of European foulbrood removed from the organ. The anterior end of the larva is shown. Fairly early stage of infection (a) showing the white bacterial mass broken into fragments as a result of the tension produced in removing the stomach contents from the organ. A somewhat later stage (b) in the course of the disease, showing the bacterial growth contained in the stomach fragmented, also the mucous or gelatinous envelope surrounding the petritrophic membrane. The stomach contents removed from a European foulbrood larva (c) about the time of its death. The bacterial growth at this time is surrounded by very little other than the peritrophic membrane. When this membrane is ruptured the contents flow out as a thin yellowish-white mass.



EUROPEAN FOULBROOD.



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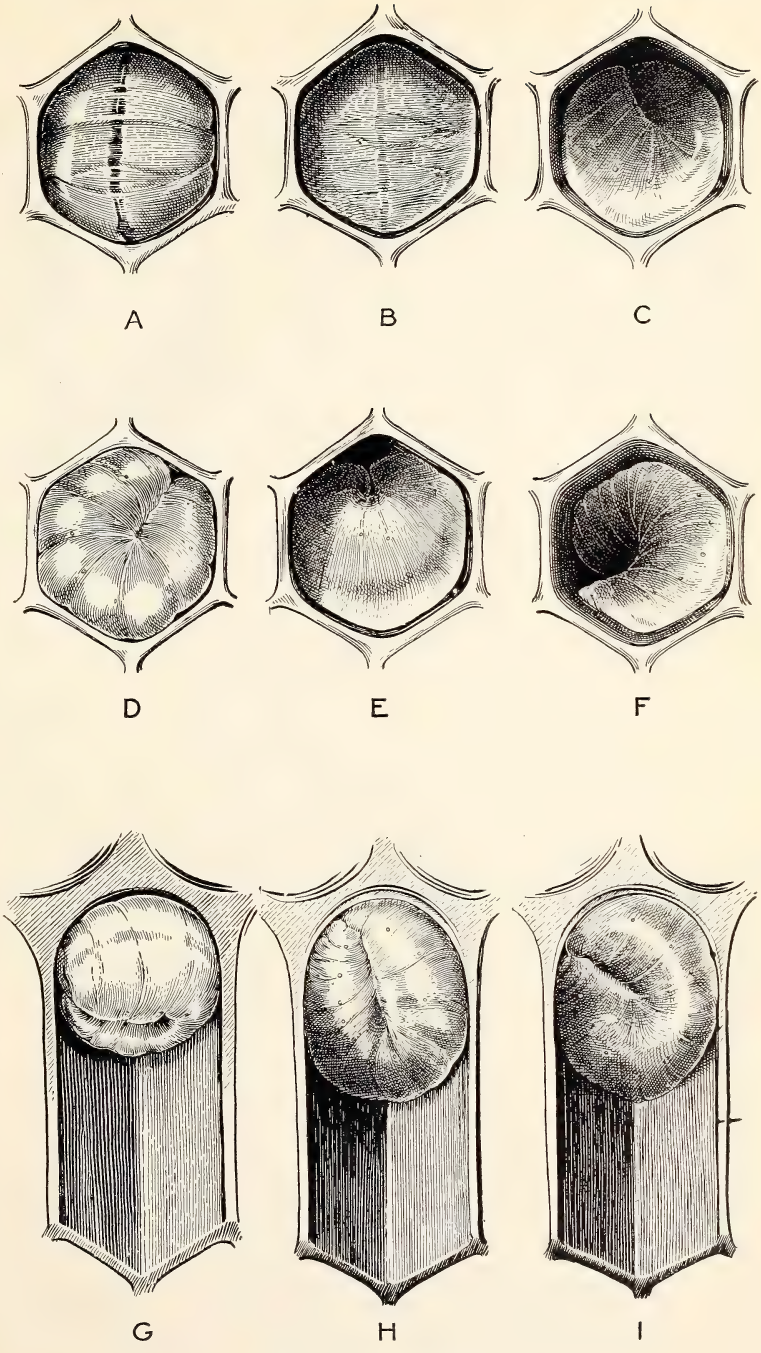

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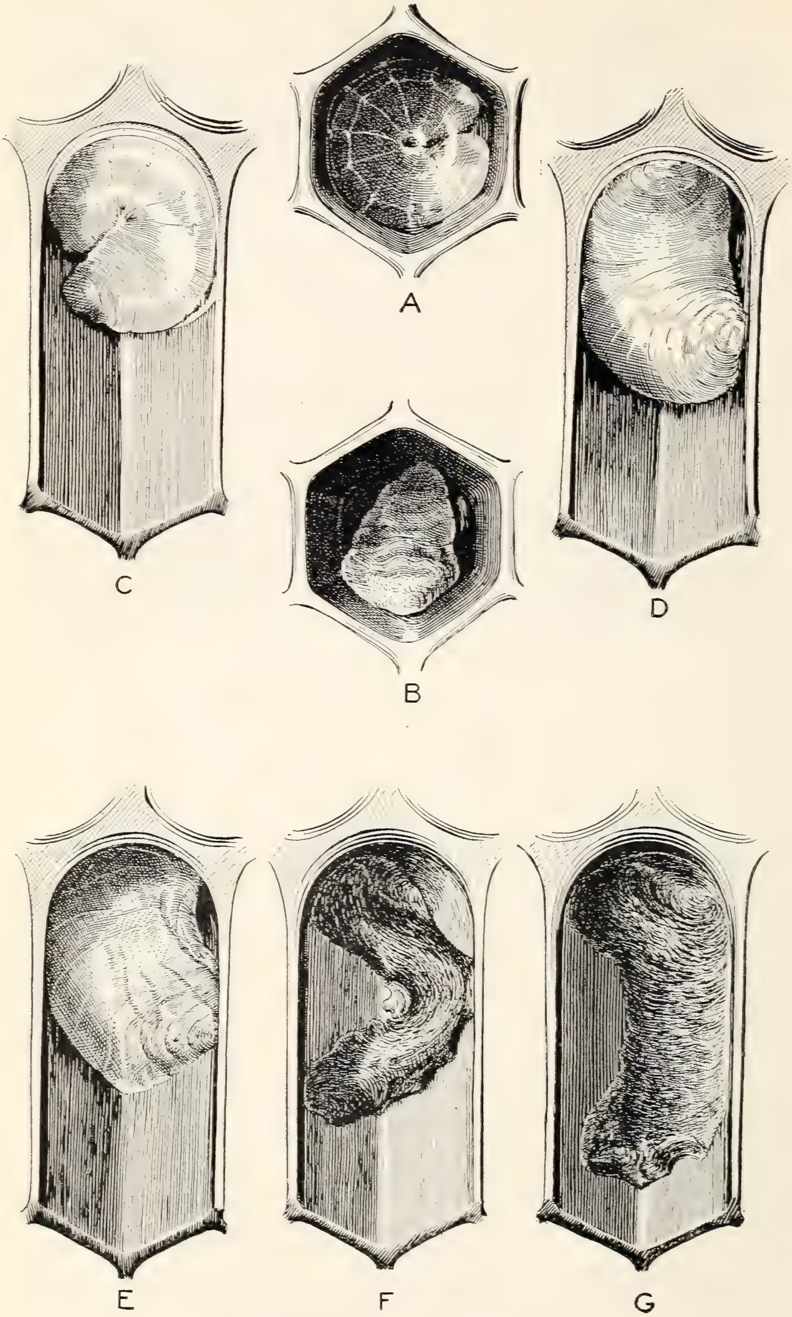
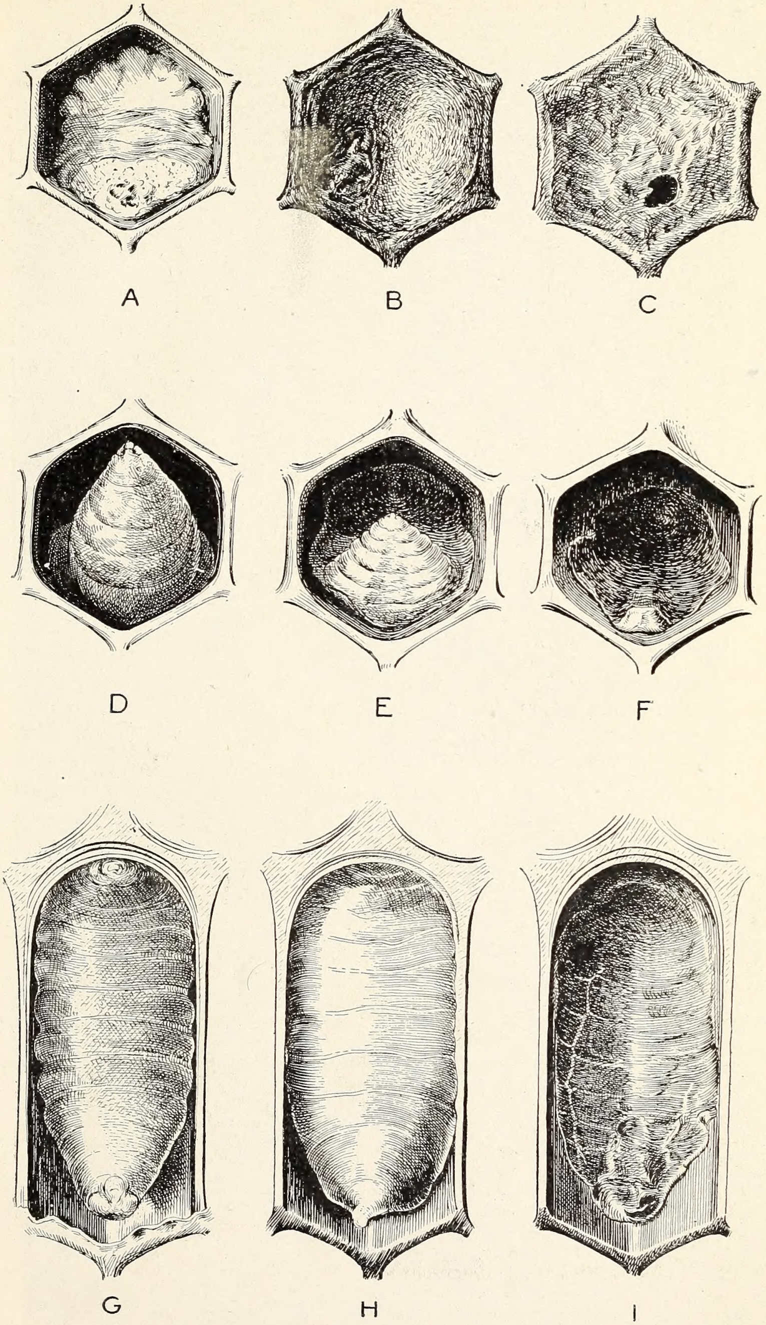


European Foulbrood.



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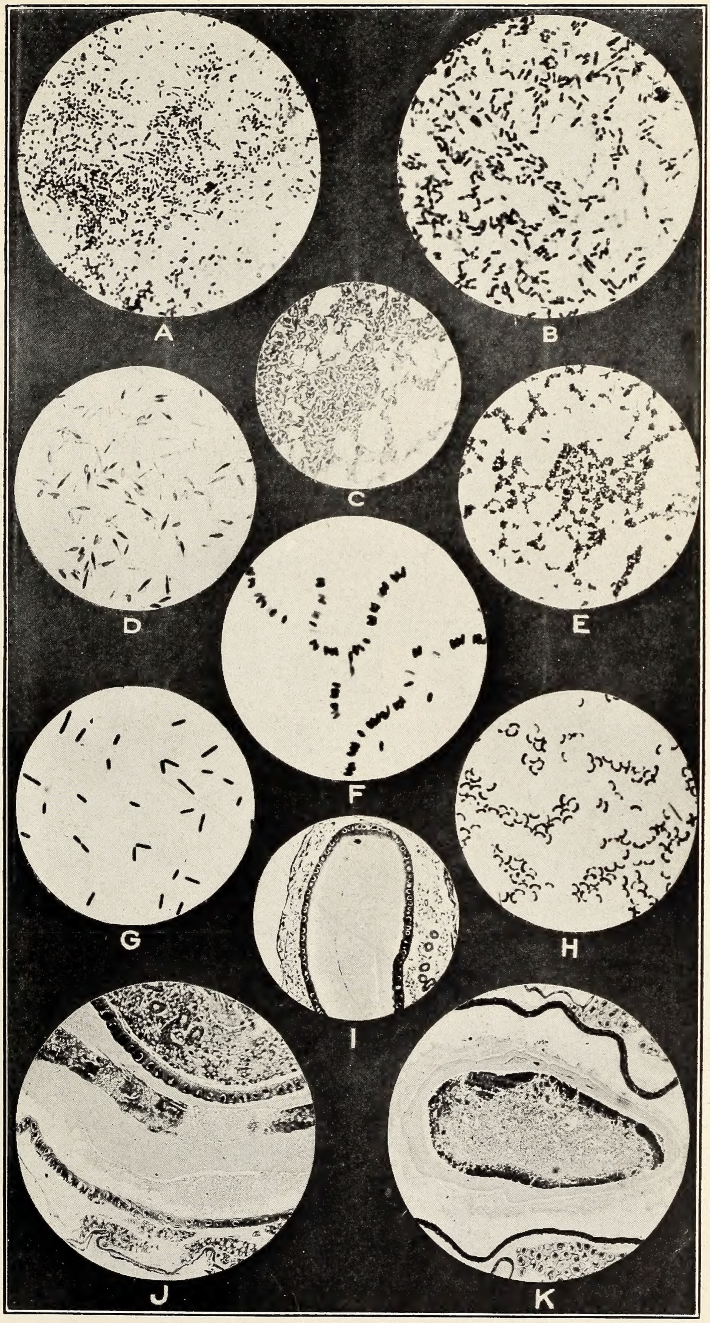

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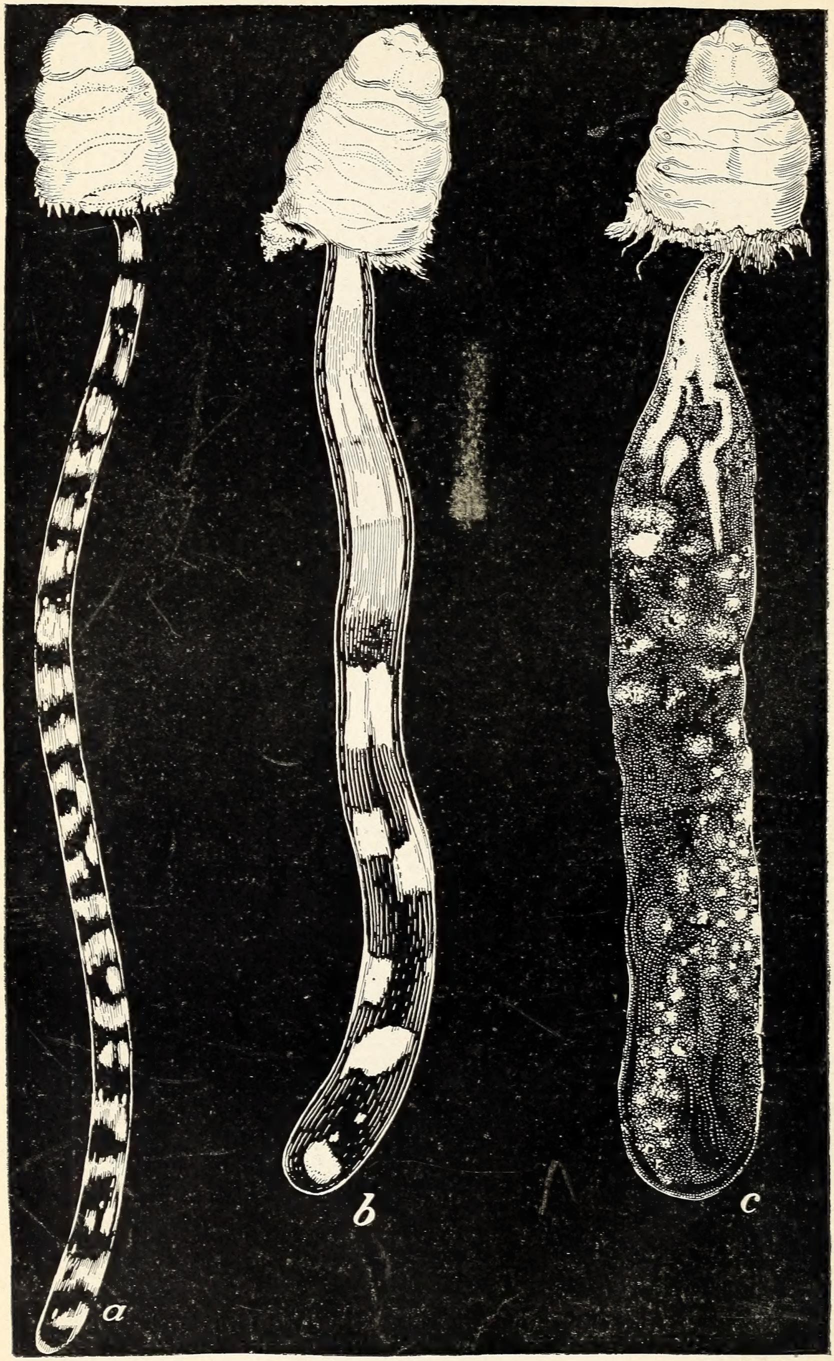

EUROPEAN FOULBROOD.